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# ADAPTIVE CHANGES INDUCED BY NATURAL SELECTION IN WILD POPULATIONS OF *DROSOPHILA*

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## INTRODUCTION

The theory of the origin of adaptations through natural selection is more than a century old, if one takes as its inception the date of Darwin's first essay written in 1842. Nevertheless, no agreement as to the rôle played by natural selection in evolution has as yet been reached. Weismann called natural selection "all powerful," but, during the first quarter of the present century, the idea fell into disrepute because of a failure to comprehend the meaning of the mutation theory and Johannsen's experiments on pure lines. So wide a divergence of opinion has been possible because the theory of natural selection has rested either on deductions from very general propositions or on inference from indirect evidence. That adaptive evolution in nature is too slow a process to be observed within a human lifetime has been taken for granted almost universally. Furthermore, selection pressures which act upon non-pathological traits of wild species have been assumed to be small.

Recent observations have shown, however, that natural populations, even of higher organisms, sometimes undergo rapid adaptive changes. Some wild species react to seasonal alterations in their environment by cyclic modifications of their genetic structure. Knowing these facts, direct observation and experimentation on natural selection has become possible. Controlled experiments can now take the place of speculation as to what natural selection is or is not able to accomplish. Furthermore, we need no longer be satisfield with mere verification of the existence of natural selection. The mechanics of natural selection in concrete cases can be studied. Hence, the genesis of adaptation, which is possibly the central problem of biology, now lies within the reach of the experimental method.

The first discovery of cyclic changes in the genetic composition of populations of wild species was made by Timofeeff-Ressovsky (1940). European as well as North American and Asiatic populations of the beetle *Adalia bipunctata* vary greatly in the elytral color pattern. Two color types, red and black, can easily be distinguished. They are known to differ by a single Mendelian gene. Near Berlin, where the species produces two and possibly three generations per year, the black type increases in relative frequency from about 37 per cent to 59 per cent from spring until autumn. During the winter the frequency of the black type is reduced and that of the red is increased. The beetle hibernates as an adult, the mortality among hibernating individuals be-

ing high. Only 4 per cent of black individuals survive hibernation, while about 11 per cent of the reds survive. The inference can be drawn that the black type is selected during the warm season.

The coloration of the Adalia beetle is a visible character easy to work with. In the corresponding Drosophila work, a character is used which is discernible only by microscopic examination of the larval salivary glands, namely the gene arrangement in the chromosomes. Variation of this character in species of Drosophila is due almost entirely to inversion of chromosome segments. Two or more such gene arrangements frequently occur in the same population. Since the carriers of different arrangements interbreed freely, some individuals have paired chromosomes with the same gene arrangements (inversion homozygotes) and others with unlike gene arrangements (inversion heterozygotes). Because the inversion homozygotes and heterozygotes are indistinguishable in external appearance, there was no reason to suppose that inversions are other than adaptively neutral characters. Not until Dobzhansky (1943, in *D. pseudoobscura*) and Dubinin and Tiniakov (1945, 1946, in *D. funebris*) found that populations which live in different habitats often differ in the relative frequencies of their gene arrangements, and that the composition of a single population may vary appreciably from season to season, was it realized that carriers of different gene arrangements may be favored or discriminated against by different environments.

The seasonal changes in the composition of the Adalia and Drosophila populations are adaptive responses of the living species to the succession of seasonal environments. It is important to note that such seasonal changes occur not only in organisms which, like the ones named above, produce several generations per year, but also in the longer lived ones. Gershenson (1945) has found changes of this sort in the hamster, *Cricetus cricetus*. Black and agouti individuals occur in Russian populations of this mammal, and the relative frequencies of these coat colors differ significantly in different seasons and in different places. The difference between the two color forms is due to a single gene.

Observations and experiments on natural selection in *Drosophila pseudoobscura* will be reviewed in the following pages. Previously published as well as unpublished data are discussed briefly; the latter will be presented in more details elsewhere.

## Local Races

Fifteen different gene arrangements are known in the third chromosome of *Drosophila pseudoobscura*. None of them occur in the entire distribution area of the species. Hence, there is no "normal" or "wild-type" gene arrangement. On the other hand, the populations of most localities contain more than one, and up to seven, gene arrangements. Because of the free interbreeding of the carriers of different arrangements, many, frequently a majority, of wild individuals are inversion heterozygotes. The population of any locality can be described in terms of relative frequencies of different gene arrangements. The frequencies may differ in populations of different localities. Sometimes the differences are more or less proportional to the distances which separate the localities. Geographic gradients or "clines" are thus formed (Dobzhansky and Epling, 1944). An example of such clines is given in figure 1.

About 50 per cent of third chromosomes in populations of south Coast Ranges of California have the so-called Standard gene arrangement, represented by black columns in figure 1. But the frequency of Standard falls to between 20 and 30 per cent in the Sierra Nevada and in the Death Valley regions which lie to the east of the Coast Ranges. Further east, in Arizona, the frequency falls to less than 5 per cent, and still further east Standard chromosomes occur but rarely. The Arrowhead gene arrangement (white columns in figure 2) is very common in

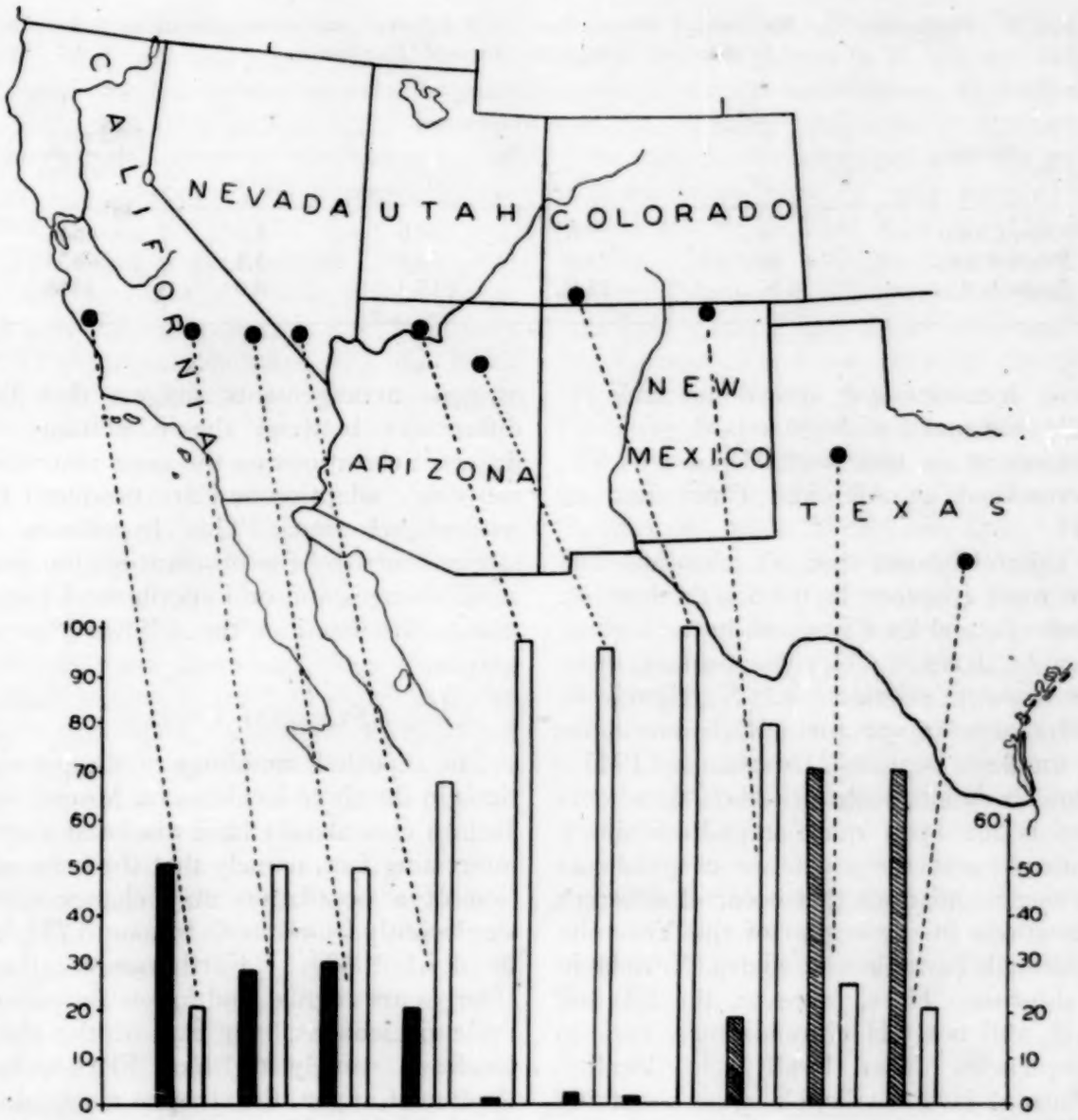


FIG. 1. Frequencies (in per cent) of Standard (black columns), Arrowhead (white columns), and Pikes Peak (hatched columns) chromosomes in populations of *Drosophila pseudoobscura* in certain localities in the western United States.

Arizona and New Mexico, so much so that populations of some localities seem to be homozygous for it. But its frequency decreases eastward as well as westward from Arizona, reaching about 20 per cent in central Texas and in coastal California. The Pikes Peak gene arrangement (hatched columns in figure 1) is common in Texas, but rapidly decreases in frequency westward.

The transect across the southwestern United States shown in figure 1 is roughly 1200 miles long. Differences in the frequencies of gene arrangements may be observed however between populations which live in localities only a dozen or so miles apart. For example, three localities on Mount San Jacinto, California, were sampled repeatedly between 1939 and 1946. The approximate distances between these localities are 10 to 15 miles. One, Keen Camp, lies at an elevation of about 4500 feet in the ponderosa pine belt, the second, Piñon Flats, lies at 4000 feet in the much drier piñon forest, and the third, Andreas Canyon, lies at 800 feet on the desert's edge. The frequencies of gene arrangements in the populations of

TABLE 1. *Frequencies (in per cent) of chromosomes with different gene arrangements in populations of localities on Mount San Jacinto (California)*

| Locality | Gene arrangements | | | | Numbers of chromosomes studied |
|---|---|---|---|---|---|
| | ST | AR | CH | Others | |
| Keen Camp | 33.7 | 23.8 | 38.0 | 4.5 | 6634 |
| Piñon Flats | 40.7 | 25.1 | 29.1 | 5.1 | 4853 |
| Andreas Canyon | 57.6 | 24.0 | 15.3 | 3.0 | 3818 |

these localities are shown in table 1. (Chromosomes with Standard gene arrangement are henceforth denoted as ST, Arrowhead as AR, and Chiricahua as CH.)

Table 1 shows that ST chromosomes are most frequent in the lowest locality, Andreas, and least frequent in the highest locality, Keen. CH chromosomes show the opposite relationship. No significant differences appear for AR chromosomes in the three localities (Dobzhansky, 1943). How common such altitudinal gradients are is an open question. Preliminary data suggest the existence of gradients among populations that occur at different elevations in the region of the Yosemite National Park, in the Sierra Nevada in California. Here, however, the ST and AR, and not CH chromosomes, vary in frequencies from locality to locality. Thus, at Jacksonville, elevation about 800 feet, 40 to 45 per cent of third chromosomes have ST and 20 to 25 per cent have AR gene arrangement. At Lost Claim Campground, elevation about 3000 feet, the frequencies of both ST and AR are between 30 and 35 per cent. At Mather, elevation 4600 feet, ST fluctuates between 20 and 40 per cent and AR between 30 and 45 per cent. Finally, at Aspen Valley, elevation about 6800 feet, ST falls to 20 and AR rises to almost 50 per cent. The frequencies of CH chromosomes are between 15 and 20 per cent in all the localities. The horizontal distance between the farthest localities, Jacksonville and Aspen Valley, is about 35 miles.

Altitudinal gradients in the frequencies of gene arrangements suggest that the differences between the inhabitants of different elevations on the same mountain range are adaptive and are produced by natural selection. This hypothesis is strengthened by the observations on seasonal changes and on experimental populations discussed in the following paragraphs.

## SEASONAL CYCLES

The repeated samplings of the populations in the three localities on Mount San Jacinto (see above) have disclosed a very interesting fact, namely that the composition of a population may change quite significantly from month to month (Dobzhansky, 1943). Furthermore, these changes are regular and follow the annual cycle of seasons. In two of the three localities, namely at Piñon Flats and at Andreas Canyon, the changes are qualitatively similar. Figure 2 gives a summary of the data for Piñon Flats. In this figure, the observations for all six years of collecting are grouped by months. It can be seen that in spring (March) the population contains about 50 per cent of ST chromosomes (shown in figure 2 by circles), and slightly more than 20 per cent CH chromosomes (shown by triangles). From March to June the frequency of ST declines to less than 30 per cent and that of CH increases to just below 40 per cent. During the summer, from June to September, the reverse change takes place, namely ST increases in frequency, and CH decreases, to about the same values which these gene arrangements had during the spring. The changes of the fre-

quencies of AR chromosomes (rectangles in figure 2) are less regular than those of ST and CH, but on the whole AR seems to follow the same path as CH. No regular changes occur in the frequency of Tree Line chromosomes (squares in figure 2).

The changes at Andreas Canyon run parallel to those at Piñon Flats. From autumn till early spring the frequency of ST chromosomes keeps on a high level, and that of CH chromosomes on a low one. From March to June ST wanes and CH waxes in frequency. During the hot part of the summer very few *D. pseudoobscura* flies can be collected at Andreas Canyon. But when the population begins to build up in numbers in September, the ST chromosomes are found to have recovered their high frequency, while CH have dwindled to about the winter level. Curiously enough, no significant changes from month to month are detectable in the Keen Camp population. Because of the climate, the breeding season of the flies is clearly shorter at Keen Camp than at the other localities, but nevertheless fly samples have been collected at Keen Camp from April to September and even October. Such time intervals are amply sufficient to detect changes in the Piñon and Andreas populations, but no cyclic changes have been found at Keen Camp (Dobzhansky, 1943).

Interestingly enough, a different kind of change has taken place in the Keen Camp population during the period of observation, from 1939 to 1946. Namely, there seems to exist a non-cyclic, or at any rate non-seasonal, trend toward decreasing frequencies of AR and CH and increasing ones of ST chromosomes. In 1939, only 28 per cent of the third chromosomes found in the Keen locality had the ST gene arrangement; in 1942 the frequency rose to 36 per cent, and in 1946 to 50 per cent. The frequencies of AR and CH chromosomes in 1939 were 30 and 38 per cent respectively. In 1946 only 15 per cent of the chromosomes were AR and only about 28 per cent CH. No such directional trends of change have appeared at Piñon Flats or at Andreas Canyon, although statistically significant differences in the composition of the populations from year to year have been recorded also in these localities (Dobzhansky, 1943, and a paper in press).

It will obviously be important to ascertain how general are the phenomena of cyclic seasonal and non-seasonal changes not only in different populations of *Drosophila pseudoobscura* but in other species as well. The fact that cyclic changes occur at Piñon Flats and at Andreas Canyon, but not at Keen Camp only some 15 miles away, makes generalizations at this time decidedly premature. Data are however available which show cyclic seasonal changes in the population of a locality in central Texas. Here the Arrowhead and Pikes Peak gene arrangements in the third chromosome of *D. pseudoobscura* are involved (Dobzhansky and Epling, 1944; cf. also figure 1). Unpublished data suggest that changes in the frequencies of ST, AR, and perhaps of CH chromosomes occur in populations of at least some of the localities in the Sierra Nevada.

## Natural Selection as a Cause of the Seasonal Changes

The regular and cyclic nature of the changes observed in the populations of *D. pseudoobscura* on Mount San Jacinto can be most reasonably accounted for by natural selection as the prime causative factor. If during the spring the carriers of CH chromosomes leave more surviving progeny on the average than the carriers of ST chromosomes, then the frequency of CH will increase and that of ST will decrease. This is what happens from March to June (Figure 2). The reversal of the change during the summer months points toward the hypothesis that, in the summer environments, the carriers of ST chromosomes survive or reproduce on the average more often than do the carriers of CH chromosomes. The ab-

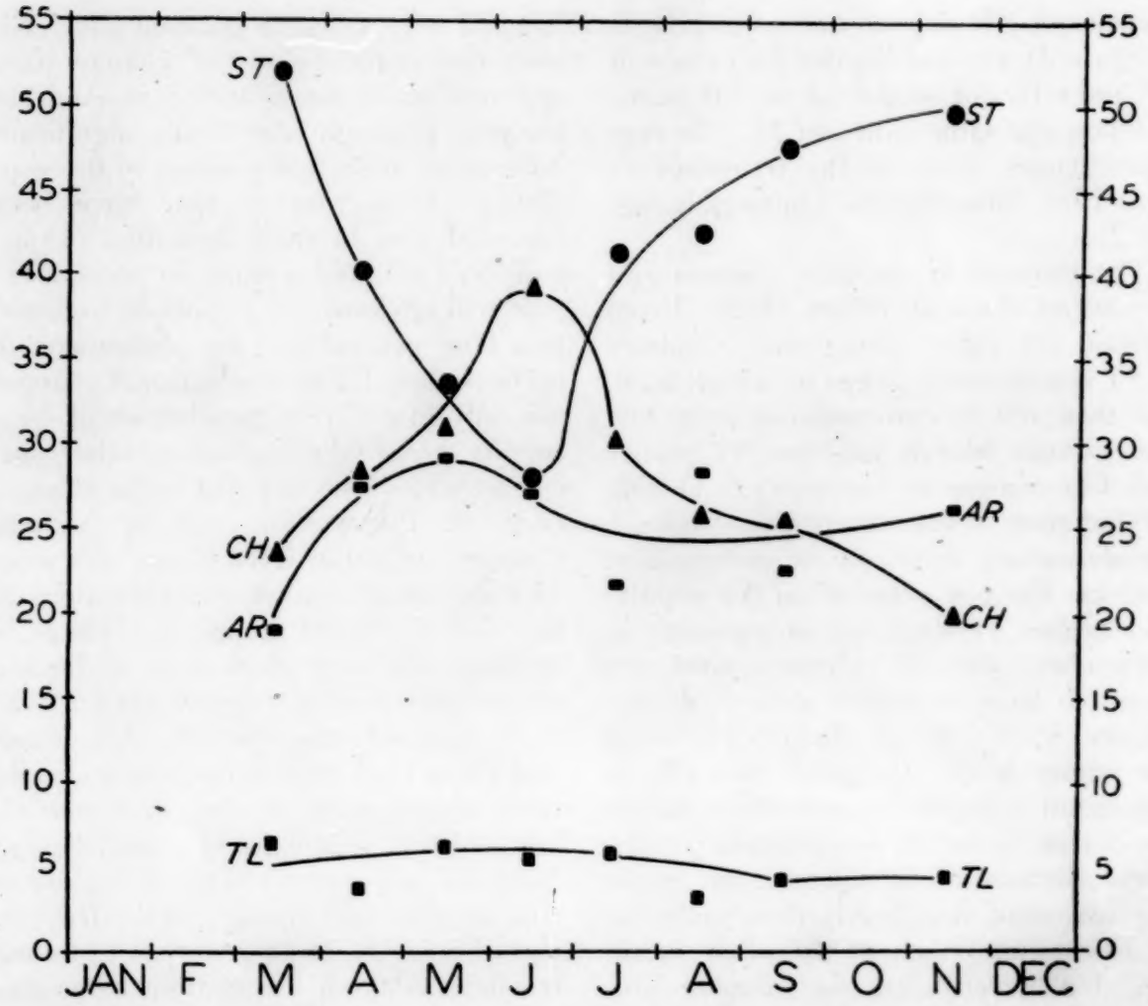


FIG. 2. Changes in the frequencies of chromosomes with Standard (circles) Chiricahua (triangles), Arrowhead (horizontal rectangles), and Tree Line (squares) gene arrangements in the population of Piñon Flats, California. Ordinate—frequencies in per cent; abscissa —months. Combined data for six years of observation.

sence of changes during the autumn and winter at Andreas Canyon suggests that flies of different chromosomal types are equivalent in reaction to the environments prevailing during these seasons.

But the great rapidity of the observed changes constitutes an apparently serious argument against accounting for them on the ground of natural selection. Indeed, at Piñon Flats the frequency of ST chromosomes falls from about 50 per cent in March to 28 per cent in June, and increases again to about 48 per cent in September (figure 2). Even though Drosophila is a rapidly breeding insect, time intervals such as these can correspond to at most two to four generations. The selective forces that are necessary to bring about changes so swift as these must be very strong.

It should be remembered however that very little is known about the intensity of selective forces which operate in natural populations. The wide-spread opinion that these forces are generally weak, and their effects negligible except in terms of quasi-geological time is only an opinion and has no basis in factual data. To find in natural populations great selective pressures and the rapid changes produced by them may be unexpected but not inherently impossible. On the other hand, the occurrence of changes does not in itself prove that they are produced by natural selection. Such proof would be very difficult to adduce from observations of

FIG. 3. A population cage.

natural populations alone. The difficulty lies in the fact that, despite persistent effort, very little has been learned as yet about the food and shelter requirements of *D. pseudoobscura* in its natural habitats. Proof of selection by a method analogous to that employed by Timofeeff-Ressovsky in Adalia is still out of the question in Drosophila.

Nevertheless, the postulated high selective advantages and disadvantages of the carriers of different gene arrangements in different environments has made practicable a still more ambitious project: to demonstrate the occurrence of natural selection by means of laboratory experiments. For this purpose, a modification of the population cage devised for Drosophila by l'Héritier and Teissier is used. These cages are wooden boxes with glass or wire screen sides and a detachable glass top (figure 3). The bottom has 15 circular openings closed by corks which carry glass containers with culture medium. Wire loops hold the containers in place (figure 4). Several hundred flies are introduced into the cage at the beginning of the experiment. These flies are a mixture of individuals with different gene arrangements in desired proportions. Within a single generation, the population of the cage increases to the maximum compatible with the amount of food given. This is usually between two and four thousand flies. The numbers of eggs deposited in a population cage are tens to hundreds of times greater than the numbers of adult flies that hatch. The competition for survival is intense. (For a more detailed description of population cage, see Wright and Dobzhansky, 1946.)

Once a month, or at other suitable intervals, samples of the eggs which have been deposited in the cages are taken, and the larvae which emerge from these eggs are grown in regular culture bottles. Salivary gland chromosomes of fully grown larvae are then examined.

It is known that many chromosomes in natural populations carry recessive lethals, semilethals, or viability modifiers. We are, however, interested not in the

FIG. 4. A cork and a jar with culture medium used in population cages.

effects of individual chromosomes on the survival of the flies, but in the selective values of ST, AR, CH, and other chromosomes as classes. In other words, in our experiments it is desired to have flies which are genetically heterogeneous, regardless of whether they are inversion homozygotes or heterozygotes. Accordingly, the initial population of a cage is always made from a mixture of several strains of each of the inversion types to be studied. As a result flies homozygous for any given individual chromosome are relatively rare in such population cages.

## SELECTIVE DIFFERENTIALS BETWEEN CHROMOSOMES FROM PIÑON FLATS

Twenty-nine experiments have been either completed or are now under way, employing populations the chromosomes of which were derived from ancestors collected at Piñon Flats. Some cages contained mixtures of ST and CH chromosomes, others of ST and AR, or of AR and CH, or of all three gene arrangements. Some were kept in incubators or constant temperature rooms at 25° C., others at 16½° C., and still others at variable room temperatures between 20° and 26° C.; some were exposed to alternation of day and night, others were kept in the dark. Two types of results have been obtained. First, at 16½° C. no significant changes in the frequencies of the gene arrangements have taken place. The relative frequencies present in the original population of the cage have been retained generation after generation. Second, at 25° C. or at room temperatures, the relative proportions of the gene arrangements have changed with time until certain definitive equilibrium proportions have been attained.

Figure 5 shows an example of changes in the frequency of ST chromosomes observed in the population cage No. 35 at 25° C. On March 1, 1946, a population was introduced into this cage containing 10.7 per cent ST and 89.3 per cent CH chromosomes of Piñon Flats origin. In about a month, in early April, the frequency of ST has approximately doubled (21.7 per cent), in early May nearly trebled (28.3 per cent), and in early June nearly quadrupled (37.7 per cent). By mid-November ST reached the frequency of 66.7 per cent and by the end of December 71.0 per cent. It can be seen, first, that the changes are greater when ST chromosomes are rare but relatively slight when they become frequent, and, second, that ST chromosomes never supplant entirely CH chromosomes. The final result of selection is a stable mixture of both ST and CH chromosomes.

Wright (in Wright and Dobzhansky, 1946) has analyzed mathematically the results of experiments like that illustrated

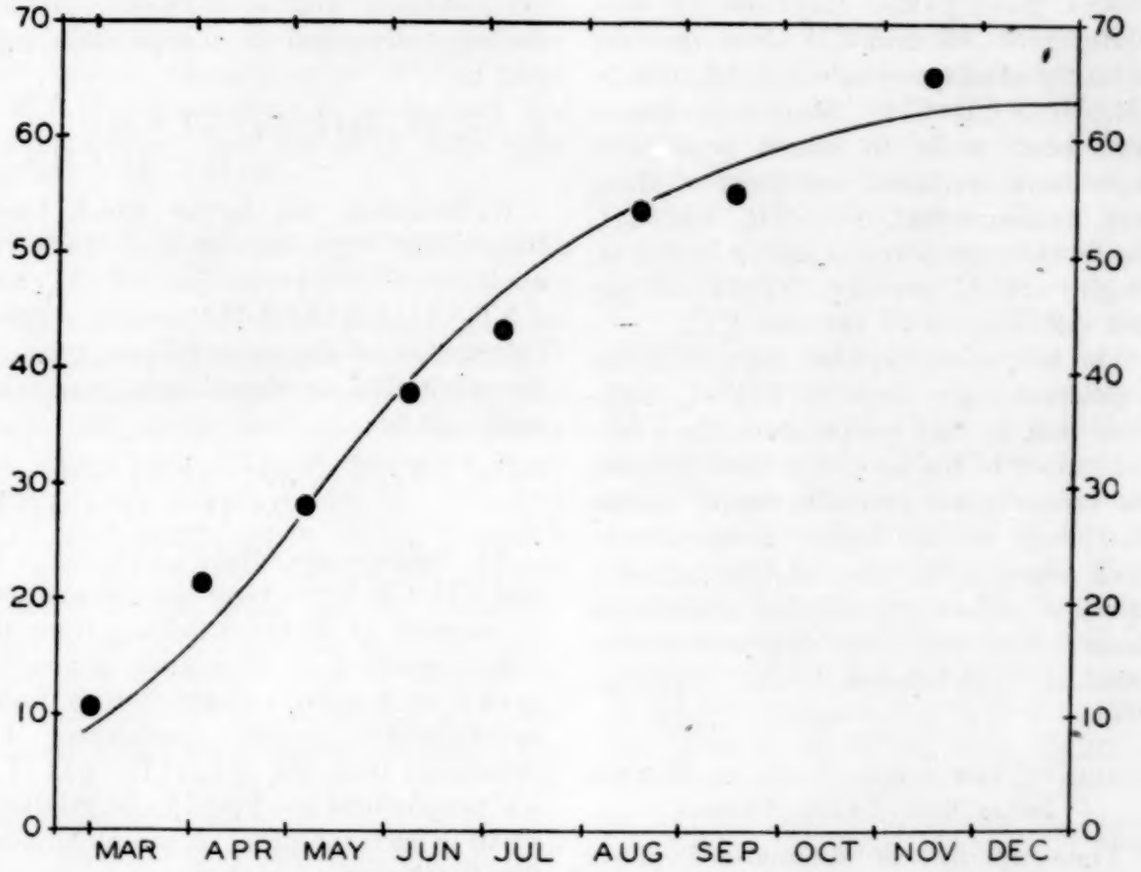


FIG. 5. Frequency of Standard chromosomes (in per cent) in different months in the population cage No. 35.

in figure 5. He has concluded that the simplest hypothesis to account for the data is that the highest adaptive value exists in ST/CH heterozygotes, and that both homozygous classes, ST/ST and CH/CH, are inferior to the heterozygotes. Furthermore, that the adaptive value of ST/ST homozygotes is higher than that of CH/CH homozygotes. The curve shown in figure 5 is calculated on the assumption that the adaptive values of the ST/CH, ST/ST, and CH/CH classes of individuals are as 1.0:0.7:0.4, and that a fly generation had an average span of one month in population cage No. 35. The observed values fit the theoretical curve remarkably well.

Since the heterozygotes, ST/CH, are superior in adaptive values to both homozygotes, the final result of selection is not elimination of either CH or ST but establishment of a certain equilibrium at which both ST and CH gene arrangements occur in the population. Since ST/ST are superior to CH/CH homozygotes, the equilibrium frequency of ST is higher than that of CH. With the adaptive values indicated above, the population would be expected to reach equilibrium at about 67 per cent ST and 33 per cent CH. The results obtained in the experiment shown in figure 5 agree with the expectation.

All the experiments in which mixtures of ST and CH chromosomes were kept at temperatures above 20° C. have led to ST becoming more frequent than CH; whenever the experiments were continued long enough, equilibria were reached at values close to 70 per cent ST and 30 per cent CH. Under similar conditions, populations containing ST and AR have reached equilibria in which ST chromosomes are also more frequent than AR. The adaptive values of the three possible genotypes must therefore be ST/AR >

ST/ST > AR/AR. Experiments with mixtures of AR and CH show that the hierarchy of adaptive values is AR/CH > AR/AR > CH/CH. Some experiments have been made in which population cages have contained mixtures of three gene arrangements, ST, AR, and CH. Equilibrium proportions are indicated at, roughly, 50–55 per cent ST, 30–35 per cent AR, and 10–15 per cent CH.

The lack of perceptible changes in the population cages kept at 16½° C. indicates that at that temperature the adaptive values of the inversion heterozygotes and homozygotes are more nearly similar than they are at higher temperatures. Such changes in the relative adaptive values of different genotypes at different temperatures have been observed experimentally (Dobzhansky and Spassky, 1944).

## Stage of the Life Cycle at Which Selection Takes Place

The experiments summarized above demonstrate that, in some environments, the adaptive values of inversion heterozygotes and homozygotes are strikingly unlike. But these experiments tell us nothing of the stage of the life cycle at which the differential survival or reproduction take place. Natural selection may operate in a variety of ways. The chromosomal types may be characterized by differential mortality, or differential longevity, or fecundity, or differences in sexual activity, or combinations of two or more of these and other variables. The adaptive value of a chromosomal type is the net effect of interaction of all the variables.

Perhaps the simplest, though by no means the only possible, hypothesis would assume a differential mortality of the different chromosomal types among the crowded larvae in the population cages. Let the frequencies of the ST and CH gene arrangements in a population cage be $q$ and $(1 - q)$ respectively. Provided that the flies mate at random with respect to gene arrangement, the proportions of heterozygotes and homozygotes among the eggs deposited in a population cage will be:

$$q^2 \text{ ST/ST} : 2q(1-q) \text{ ST/CH} : (1-q)^2 \text{ CH/CH}.$$

If, however, the larvae which hatch from these eggs survive and reach the adult stage in a proportion 0.7 ST/ST : 1 ST/CH : 0.4 CH/CH, then the relative frequencies of the chromosomal types of the adult flies developed in a population cage will be:

$$0.7\ q^2 \text{ ST/ST} : 2q(1-q) \text{ ST/CH} : 0.4\ (1-q)^2 \text{ CH/CH}.$$

The frequencies of the ST/ST, ST/CH, and CH/CH types have been determined in samples of larvae hatching from the eggs deposited in population cages but grown in regular culture bottles under approximately optimal conditions. The deviations from the $q^2 : 2q(1-q) : (1-q)^2$ proportions are found to be relatively small in such samples. A sample of adult flies hatched in a population cage was now taken, and the chromosomal constitution of these flies was determined with the aid of a suitable method (Dobzhansky, 1947b). The numbers of ST/ST, ST/CH, and CH/CH flies which would be expected to occur in this sample if there were no differential mortality between the egg and the adult stage were calculated with the aid of the Hardy-Weinberg formula $q^2 : 2q(1-q) : (1-q)^2$. The observed and the expected values are as follows:

| | ST/ST | ST/CH | CH/CH |
|---|---|---|---|
| Observed | 57 | 169 | 29 |
| Expected | 78.5 | 126.0 | 50.5 |
| Deviation | −21.5 | +43.0 | −21.5 |

Among the adult flies, the heterozygotes, ST/CH, are considerably more frequent, and the homozygotes less frequent, than expected on the basis of the Hardy-Weinberg formula. Since, as stated above, the Hardy-Weinberg proportions

are approximately realized among the eggs deposited in the population cages, a differential elimination of ST/ST and CH/CH homozygotes at some time between the egg and the adult stage may be regarded established.

### Experiments on Chromosomes of Different Geographic Origin

The above described experiments on the behavior of chromosomes with different gene arrangements in population cages have been made with flies the ancestors of which were collected at Piñon Flats, Mount San Jacinto. The ST, AR, and CH gene arrangements occur, however, in populations from most of the western United States, from British Columbia, and from Lower California. The question that naturally arises is whether or not the biological properties of these chromosomes are constant throughout the geographic area in which a given gene arrangement occurs (Mayr, 1945).

Population cage experiments are now in progress on mixtures of flies with different gene arrangements from Keen Camp and from Mather, California. These localities are respectively about 12 and 300 miles from Piñon Flats. The data so far obtained leave no doubt in that the adaptive properties of chromosomes with the same gene arrangement but of different geographic origin may be different. The relevant data will be published in more detail later; the following comparison will suffice as an illustration.

On March 2nd, 1946, population cage No. 36 was started with a mixture of about 12 per cent ST and 88 per cent AR chromosomes of Piñon Flats origin. In late November of the same year this population cage contained about 67 per cent ST and 33 per cent AR. It is obvious that when populations of ST and AR of Piñon Flats origin reach equilibrium, ST chromosomes are decidedly more common than AR. This is confirmed by the experiment No. 34, in which the initial population of a cage consisted on February 25th, 1946, of 85 per cent ST and 15 per cent AR. By late summer and autumn of the same year the frequency of AR in this cage fluctuated between 20 and 25 per cent AR. But the results of experiments on ST and AR chromosomes of Mather origin are quite different. On December 22nd, 1945, population cage No. 29 was started with about 70 per cent ST and 30 per cent AR chromosomes from Mather. In late June and late July of 1946, when the experiment was terminated, the frequency of ST had fallen to 54–55 per cent, and that of AR had risen to 45–46 per cent. In a parallel experiment started simultaneously, the initial population of cage No. 32 contained 19 per cent ST and 81 per cent AR chromosomes of Mather origin. By late summer and autumn of 1946, the frequency of ST had risen in this cage to 50–54 per cent, and the frequency of AR had fallen to 46–50 per cent (figure 6). It seems safe to conclude that mixtures of ST and AR chromosomes of Mather origin reach equilibria at about 50–55 per cent ST and 45–50 per cent AR. This is significantly different from the behavior of ST and AR chromosomes of Piñon Flats origin. All the experiments described in this paragraph were carried out in a temperature-controlled room at 25° C.

Chromosomes with the same gene arrangement but of different geographic origin may, then, behave differently. This fact has a bearing on the problem of the genetic nature of the adaptive differences between chromosomes with different gene arrangements. The properties of a chromosome may, in general, depend on its gene contents, or on the gene arrangement itself (position effect), or on a combination of both factors. The different behavior of the ST and AR chromosomes of Piñon Flats and Mather origin proves that the gene contents of these chromosomes are different. We are evidently dealing with gene complexes evolved under the control of natural selection which confer upon their carriers ecological properties which adapt them to

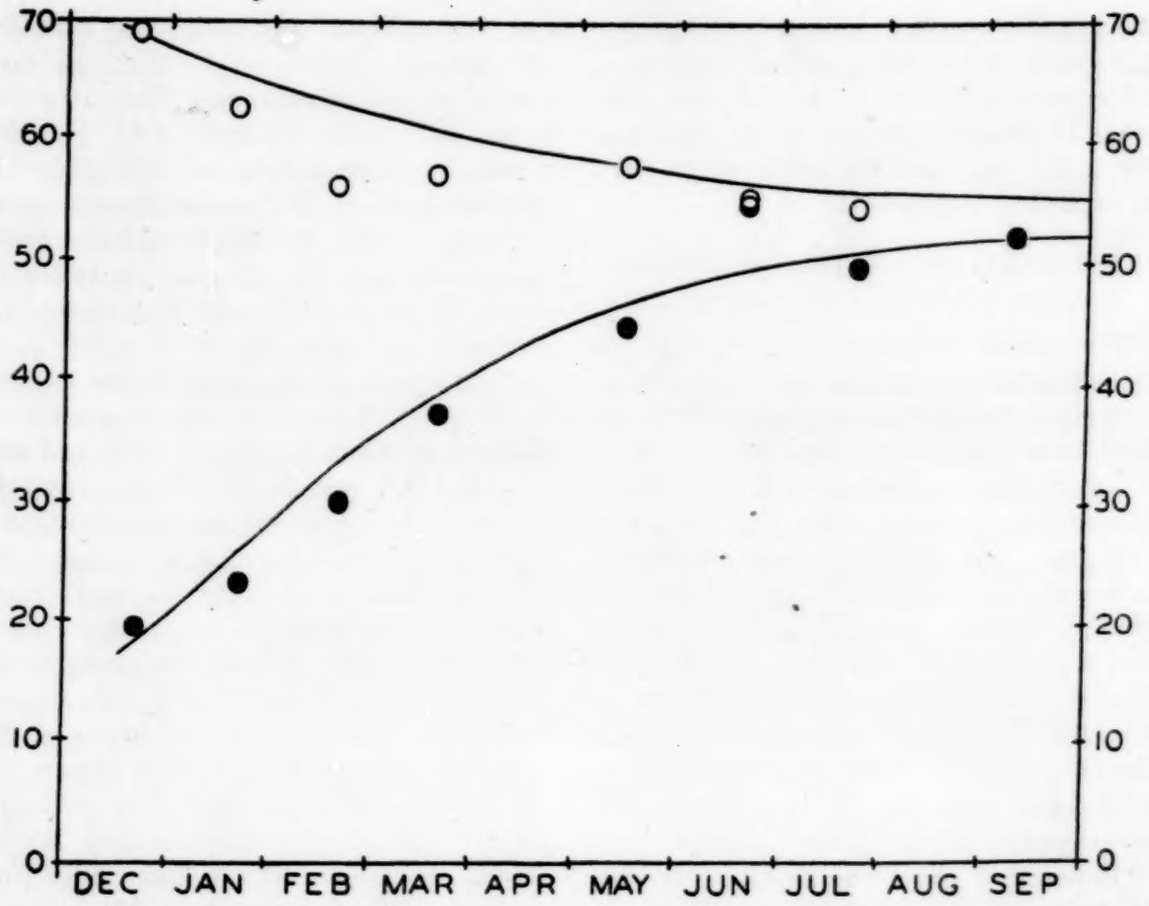


FIG. 6. Frequency of Standard chromosomes (in per cent) in different months in the population cages Nos. 29 (white circles) and 32 (black circles).

certain seasonal and local environments. Since inversions modify the gene arrangement and diminish the effective crossing over between the chromosomes involved, their biological function is binding together of the gene complexes of proven adaptive worth. In this sense, the effect of natural selection on the chromosomal inversions is indirect. For the purposes of this discussion, it must be borne in mind that the inversions may be simply structural characters marking chromosomes with different gene contents. Of course, the possibility still exists that the biological properties of chromosomes with different gene arrangement differ not only because of the dissimilarities of their gene contents but because of position effects as well.

## HETEROSIS

In the experiments discussed above, the populations of the cages reached, or tended to reach, equilibria at which all the gene arrangements introduced into a cage at the beginning were preserved in certain proportions in the final population. Natural selection does not lead to the complete elimination of some and to fixation of other gene arrangements. The final outcome of the selective process is the establishment of those relative frequencies of the competing gene arrangements at which the average adaptive level of the population as a whole is the highest one attainable. This outcome of the selective process is explained by the fact that inversion heterozygotes, ST/CH, ST/AR, and AR/CH, possess adaptive values greater than the corresponding homozygotes ST/ST, CH/CH, and AR/AR. The populations at equilibrium contain the greatest possible proportions of the well adapted heterozygotes compatible with the lowest possible propor-

tions of the relatively ill adapted homozygotes.

The superiority of the heterozygotes over the homozygotes is indicated in all but one experiment carried out at temperatures above 20° C. The single exceptional experiment, No. 31, was started in December 23, 1945. The initial population of the experimental cage contained 45 per cent of ST chromosomes and 55 per cent of chromosomes with the Tree Line gene arrangement, all the chromosomes descended from flies collected at Mather, California. Tree Line is a gene arrangement rather widespread geographically but relatively rare in most natural populations. In the population of Mather its frequency is approximately 10 per cent of the total. The changes in population cage No. 31 were rapid. Within a single month, in late January of 1946, the frequency of Standard chromosomes had risen to 62 per cent, while Tree Line fell to 38 per cent. By mid-May of 1946 the corresponding frequencies were 82 and 18 per cent, in late July 93 and 7 per cent, and in mid-November 99 and 1 per cent. It seems clear that no equilibrium is being reached in cage No. 31; instead, the Tree Line chromosomes were being eliminated and entirely supplanted by ST chromosomes. This is possible only because ST/ST homozygotes seem to have an adaptive value higher not only than Tree Line homozygotes but also than Standard/Tree Line heterozygotes.

The Tree Line gene arrangement seems to be deleterious to its carriers in homozygous as well as in heterozygous condition. The question naturally arises: why are Tree Line chromosomes retained in natural populations rather than eliminated by natural selection? A possible answer is provided by experiment No. 33, as yet uncompleted. The initial population of this cage, started on January 16th, 1946, consisted of 34 per cent Arrowhead and 66 per cent Tree Line chromosomes of Mather origin. Within a month, in mid-February, the proportions had changed to 58 and 42 per cent respectively. The frequencies of AR chromosomes rose to 69 per cent in mid-April, 75 per cent in mid-July, and 80 per cent in early November. It appears that, in this experiment, the Tree Line chromosomes are not eliminated entirely; instead, an equilibrium at about 80 per cent AR and 20 per cent Tree Line chromosomes is indicated.

Arrowhead/Tree Line heterozygotes are, under the conditions of this experiment, superior to both Arrowhead and Tree Line homozygotes. This explains not only the fact that the Tree Line gene arrangement is relatively rare but that it is nevertheless retained in natural populations. Tree Line chromosomes form adaptively valuable heterozygotes with AR but not with ST chromosomes present in the same populations. This assures the retention, but prevents excessive increase of the incidence, of the Tree Line gene arrangement in the species.

The classical theory of heterosis assumes that the restoration of vigor following intercrossing of inbred lines is due to the covering up of deleterious recessives by favorable dominants. The greater vigor observed in inversion heterozygotes as compared with homozygotes requires a different explanation. Inversion homozygotes found in natural populations of Drosophila are evidently no more "inbred" than are the heterozygotes in the same population. The lower adaptive value of homozygotes is, therefore, not due to manifestation of deleterious recessives normally covered up by their more favorable dominant alleles. The higher adaptive value of heterozygotes is, in this case, a product of the action of natural selection on the heterozygous genotypes, which are more widespread in natural populations than inversion homozygotes. In view of their greater frequency, the adaptive qualities of heterozygotes may be maintained by natural selection on a level higher than that in homozygotes.

## Adaptation and Plasticity

The conflict between adaptive fitness and genetic variability was pointed out by Haldane (1937), Dobzhansky (1937, 1938), and Mather (1943). The mutation process furnishes the raw materials without which adaptive changes cannot be constructed, but the same process also unavoidably produces a multitude of poorly adapted variants. Restriction of the supply of heritable variability might permit a species to reach a higher level of immediate fitness, but it jeopardizes its adaptability to changing environments. When an adaptive change does occur it generally uses up a part of the available supply of variability and thus limits the possibilities of further change.

Observations and experiments on the gene arrangements in third chromosome of *Drosophila pseudoobscura* demonstrate that a remarkable adaptive mechanism exists in this species. The plasticity of the species is so great that it reacts by adaptive reconstructions of the genotype to environmental changes of even so ephemeral a nature as the succession of the year's seasons. There is no doubt that environmental changes in time and in space elicit adaptive responses of a more durable kind as well. And yet all the responses occur without expenditure of genetic variability stored in the populations. This great efficiency of the adaptive mechanism is made possible by the fact that the inversion heterozygotes are endowed with survival values greater than the inversion homozygotes.

We have seen that in the Piñon Flats population the frequencies of ST chromosomes increase and those of CH chromosomes decrease during the hot part of the summer (figure 2). In summer environments the adaptive values of the carriers of ST chromosomes are evidently higher than those of CH. Yet, during the spring months the carriers of CH seem to be superior to the carriers of ST chromosomes. An uncommonly hot or prolonged summer can not eliminate all the CH chromosomes and thus endanger the welfare of the species during the ensuing spring. This is because the completion of the selective process in the summer environment would lead not to elimination of CH chromosomes but to establishment of a certain equilibrium of ST and CH gene arrangements. The genetic variability is, therefore, preserved intact and the species remains capable of immediate and rapid response to new environmental changes. This resembles the balanced polymorphism studied by Fisher (1930) and Ford (1940).

Adaptive mechanisms which permit very rapid reconstructions of the population genotype in accordance with the demands of the seasonal environment will probably be most important and widespread in species which inhabit the temperate and frigid climatic zones, as well as tropical territories in which great seasonal changes regularly occur. Inversion systems in which the heterozygotes possess adaptive values higher than the homozygotes are capable of responding very rapidly to alterations in the environment. The biological significance of heterozygosis for inversions is, however, not necessarily confined to seasonal changes. The high adaptive value of inversion heterozygotes may be desirable because it permits rapid adjustments to various microenvironments which are found in the same region and which are not connected with seasonal phenomena. Thus, inversions may be found also in tropical countries characterized by relatively invariant climates. Indeed, fragmentary data now available show that inversion heterozygotes occur very frequently in natural populations of some tropical species of Drosophila (Pavan, 1946, and unpublished data of the writer). Inversion heterozygosis may be now connected with seasonal changes also in some temperate zone species. Carson and Stalker (1947) found no seasonal changes in frequencies of inversions in *D. robusta* near St. Louis, and the present writer has ground to suppose that such changes

are absent in populations of *D. persimilis* in the Sierra Nevada of California. The inversion mechanism may facilitate adaptation to different ecological niches within the same geographic environment. Here is a promising field for further studies.

## Summary

The gene arrangement in the third chromosome of *Drosophila pseudoobscura* is variable. Each gene arrangement occurs in populations of a definite geographic area. However, two or more gene arrangements may occur together in many populations. Inversion homozygotes and heterozygotes occur frequently in natural populations.

The relative frequencies of various gene arrangements in some populations undergo seasonal cyclic changes. These changes are produced by natural selection, and represent adaptive reconstructions of the population genotype, thus facilitating survival in different seasonal environments.

Some of the changes taking place in nature can be reproduced experimentally in "population cages" (figure 3). Populations containing desired proportions of chromosomes with different gene arrangements are introduced into the cages, samples of the eggs deposited by the flies in the cages are taken from time to time, and the incidence of the chromosomes of different types determined in these samples. Most experiments have been made with chromosomes derived from parents collected at Piñon Flats, Mount San Jacinto, California, and some from Mather, California.

The relative proportions of chromosomes with different gene arrangements remain constant in population cages kept at 16½° C. Changes are frequently observed in cages kept at temperatures above 20° C.: the incidence of some gene arrangements increases and of others decreases. However, the final outcome of the selective process is rarely a complete replacement of one gene arrangement by another. Instead, an equilibrium is usually reached at which all gene arrangements present in the initial population of an experimental cage are retained, but often with frequencies very different from the initial ones. The establishment of equilibria in the populations indicates that individuals heterozygous for different gene arrangements (inversion heterozygotes) are characterized by the highest adaptive values, while homozygotes are relatively inferior in survival and reproduction. The correctness of this interpretation is demonstrated by means of observations on deviations from the Hardy-Weinberg proportions of heterozygotes and homozygotes among the adult flies developed in the population cages (see page 10).

The balanced polymorphism, resulting from the superiority of the heterozygotes over the corresponding homozygotes, permits the species to react adaptively to changes in its environment. The species is "buffered" against environmental change; at the same time, the adaptive responses do not consume or deplete the store of hereditary variability present in the species.

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# SEXUAL BEHAVIOR AND ISOLATION IN DROSOPHILA. I. THE MATING BEHAVIOR OF SPECIES OF THE *WILLISTONI* GROUP

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Species maintain their distinctness from other related species by means of isolating mechanisms. Divers analyses of the isolating factors show them to be of sundry types operating at different levels and in various manners in the lives of the organisms. These mechanisms function and thereby become identifiable only when species come into contact. Obviously these isolating factors must have originated before this happens, or the species could not maintain their integrity. This indicates that it is very improbable that the student will ever see any of these isolating mechanisms come into existence. Their origin must therefore be studied by indirection.

Of the various subdivisions of reproductive isolation that are to be found actively functioning in nature, sexual isolation appears to be one of the most effective. Most bisexual animals court and mate only with individuals of their own species; at the same time, they are able to disregard specimens of other closely related species that they happen to meet. Such discrimination seems to depend upon innate abilities of the organisms which express themselves through a behavioral pattern that ensures sufficient successful matings for the survival of the species and simultaneously isolates the species.

The investigation of the sexual behavior of a group of closely related species should not only furnish information concerning the specific behavioral mechanisms involved, but also such findings might be of general value in the whole problem of the nature of behavioral mechanisms. Such a study demands as a first step a detailed inventory of the whole set of actions and reactions involved in the sexual behavior. The cataloguing of such behavioral activities is of more than purely descriptive value since the degree and kind of differences between the species are measures of the relationships of the various species. These behavioral differences can then be compared with the morphological differences with the view of determining whether or not the two types of differences parallel or diverge from each other. Finally, such a study gives us an acquaintanceship with and possibly clues to those specific activities that are the actual isolating mechanisms.

For a variety of reasons, most studies of this type can not be done exclusively in the field but must be at least partially conducted in the laboratory. This necessitates the selection of material that can adequately be handled under laboratory existence without imposing upon the organisms conditions that patently call forth abnormal reactions. Various workers have used sundry materials, but none seems to be more suitable for such studies than members of the genus Drosophila. Not only are they amenable to laboratory conditions, but there is also known a great deal about their biology, thanks to the work of many investigators. In addition, the work of the past decade has shown that there are within the genus many groups of closely related species which offer ideal material for an analysis of sexual behavior.

The present study concerns itself with the *willistoni* group of the subgenus Sophophora of the genus Drosophila.

The known species of this group are *nebulosa* Sturtevant, *fumipennis* Dobzhansky and Pavan, *capricornis* Dobzhansky and Pavan, *sucinea* Patterson and Mainland, *paulista* Dobzhansky and Pavan, *willistoni* Sturtevant, and *equinoxialis* Dobzhansky. Morphologically *equinoxialis* and *willistoni* are almost indistinguishable (Dobzhansky, 1) while *paulista* and *sucinea* are both closely related to *willistoni* and to *equinoxialis,* the four species thus forming a compact subgroup. *D. fumipennis* and *nebulosa* are quite distinct from each other and also from the other four. Finally, *capricornis* seems to be intermediate between *willistoni* and *fumipennis.* Thus a graded series of morphological distinctness exists between the various members of the group, indicating that the species have differentiated morphologically in various degrees from the ancestral stock from which they have descended. All of the species are reproductively isolated, not only in nature but also under experimental conditions. In this connection, it should be observed that reproductive isolation which is rigid in nature will sometimes break down under the experimental conditions of the laboratory.

Of greatest interest from an evolutionary aspect is the fact that two of the species (*equinoxialis* and *willistoni*) are completely isolated reproductively (Dobzhansky, 1) from each other even though they appear to be morphologically almost indistinguishable.[1]

The present study records data derived from observing intraspecific matings between pairs of all species of the group except *paulista.* (Stocks of *paulista* are no longer extant.) Data on the observations of interspecific pairings, certain physiological data and other information gained from special experiments are withheld for future publications. Various authors (Sturtevant, 8, 9, 10; Stalker, 7; Lancefield, 3; Dobzhansky and Koller, 2; Mayr and Dobzhansky, 5; Mayr, 4; Wallace and Dobzhansky, 11, and Rendel, 6) have described the courtships of numerous species of Drosophila, but in the *willistoni* group the mating behavior of only *nebulosa* and *willistoni* has been previously recorded (Sturtevant, 8).

[1] The females can be separated on the basis of the shape of the inner duct of the dorsal spermatheca.

## Materials and Methods

Samples of all stocks except that of *equinoxialis* were supplied from the Columbia University collection. The *equinoxialis* stock was contributed by the University of Texas, which in turn had received it from Columbia University. The geographical localities at which the stocks had been originally collected are as follows: *equinoxialis,* Teffe, Brazil; *willistoni,* Belem do Para, Brazil; *sucinea,* Jalapa, Mexico; *capricornis,* Jacarepagua, Brazil; *nebulosa,* Belem do Para, Brazil; *fumipennis,* Cantareira, São Paulo, Brazil. The cultures were reared at temperatures of 19°–23° C. From these cultures virgin individuals were collected and isolated in shell vials which contained a small amount of food medium. Vials containing isolated individuals were kept under the same conditions as the rearing cultures. Either one or two individuals of the same sex were isolated in each vial and, after they had been aged in isolation for from one to twenty days, individuals of both sexes were introduced into a small observation cell of approximately 2 ccm. volume and observed under 10 × magnification. Two types of cells were used. The first consisted of a top and bottom of thin plate glass with a circular vertical wall of beeswax. A beeswax plug was used to close the opening (Mayr, 4). The second type of cell had glass walls as well as glass tops and bottoms, with the bottom coated with a thin layer of beeswax. A beeswax stopper was also used for this cell. Specimens were introduced into the cells directly from the isolation vials without being etherized. Despite careful handling, this involved some agitation of the individuals and thus irritation. The temperature of the cells

varied from day to day while the observations were being conducted. The total range was from 21° to 29° C. with most of the observations being conducted within the 23°–26° C. range. Successful copulations were observed at all temperatures encountered. Copulations did not terminate all observation periods for if the individuals ignored each other or had not copulated at the end of twenty to thirty minutes after introduction into the cell, they were usually returned to their respective isolation vials. Often, however, the pairs were observed for longer periods of time and especially in the case of *equinoxialis*. Two pairs of this species were observed continuously for eleven hours without copulation occurring. The standard procedure was to observe pairs of this species for at least one hour or longer.

## Terminology

Sturtevant (8, 9) has described and Mayr (4) restated those elements of behavior that are exhibited most constantly by Drosophila males. They are as follows: vibrating, waving, scissoring, licking, and circling. In addition, the following elements are found within the group:

1. *Tapping.* Rendel (6) first described this activity in his observations on *D. subobscura* and, although he does not say how the male approaches the female, the similarity of the action to that found in the *willistoni* group is so great that it should be considered by the same name. The male normally approaches head-on the side of another individual, either male or female, and taps the side of the other fly with his fore legs. This is the usual manner by which courtship is initiated in all members of the *willistoni* group.

2. *Upper-cutting.* The male, while standing close to and facing the side or rear of the female, strikes upwards with his fore legs. The motion involves the head which jerks upward at the same time. The legs strike the side of the female's body and the under surface of the wing, while the head of the male may strike the under surface of the female's wing. The motion is most obvious when males of *sucinea, capricornis, willistoni* and *equinoxialis* are courting reluctant females.

3. *Stamping.* An excited male, having gone through the initial phases of courtship and being in position ready to mount the female, will often rapidly stamp his fore legs when the female fails to spread her wings.

The females of *Drosophila* possess methods by which they can repel and avoid the courting males. Mayr (4) has indicated that non-receptive females of *pseudoobscura* and *persimilis* avoid the courting males by walking away, wing-flicking and depressing the abdomen or a combination of these methods. Females of the *willistoni* group do not employ wing-flicking or abdomen-depressing to repel the males. Males, however, utilize wing-flicking, often violently, to repel other males that are courting them. Within the *willistoni* group, the following methods are utilized by non-receptive females to repel courting males:

1. *Extruding.* The female extrudes the genitalia, a method which enables her to repel the male and at the same time continue feeding without interruption. If the male persists in courting, the extrusion may become more pronounced and at the same time the female will elevate the posterior part of her body. In extreme cases this involves the whole body, and females of *capricornis* and *fumipennis* seem almost to stand on their heads. A highly irritated female will extrude whenever any and all individuals come within close proximity. There is considerable individual difference and some specimens seem not to be able to extrude nearly as much as do others. A female does not extrude and at the same time walk, run, or fly.

2. *Decamping.* The females escape from the immediate area of the male by running, walking, or flying.

3. *Ignoring.* The female simply sits still.

Finally, the activity known as *sparring* should be noted. This seems almost universal throughout the genus. Two individuals, regardless of sex, when they pass close to each other will extend their middle legs and spar with each other. The female, while in copula, will utilize the action to repel other males. Rendel (6) and Wallace and Dobzhansky (11) have used this term as a synonym of tapping, i.e., as an activity of the fore legs, but to avoid confusion it would seem best to restrict sparring to the fencing type of movements of the middle legs.

## Initial Contact Between Individuals

In nature all bisexual animals face the problem that the individuals of opposite sexes must find each other in order that courtship and copulation may take place. There are many mechanisms, some of them very specific, whose actions facilitate the meeting of the two sexes. In Drosophila the main mechanism seems to be that the adults are attracted to food upon which they feed, and since the food materials (decaying vegetable materials, decaying fruits, sap, fungus, and flowers) occupy at any given time relatively small areas, the insects that are attracted to them are brought into relatively close proximity. Further, the young are raised upon the same type of material as that upon which the adults feed; thus, if a given unit of food supply stays in existence for a considerable period of time, then the young flies can mature, mate and breed in the same small area in which they had grown.

If individuals of the *willistoni* complex are observed, either in the pint culture bottles in which they are reared, or in observation or isolation cells, it will be noted that waves or surges of moving about occur. An individual may remain in one place, feeding, cleaning, or just sitting still for a time, and then suddenly will start walking or running about. After a short period of moving about, it then settles down again and is quiet for an interval. The moving about gives rise to two results: (1) the moving individual passes so close to another individual that sparring contact occurs; (2) the movement causes the moving individual to be seen by a resting male which then runs up and taps the moving individual, regardless of its sex. Most Drosophila can and do mate in the dark, and under such conditions it seems probable that the initial courtship contact is due to chance encounter resulting from the surges of movement. Under lighted conditions, however, there is no doubt that members of the *willistoni* group do see each other and that a resting male will actually "tense" when another individual moves within his near vicinity. Some species are much more active than others, and in the *willistoni* group *willistoni* and *nebulosa* are by far the most active while *equinoxialis* and *fumipennis* are the most lethargic. In addition, *nebulosa* has the habit of constantly raising and dropping its wings which seems to act in the same manner as locomotion in stimulating other individuals.

Thus the initial contact between individuals of the two sexes seems dependent in the *willistoni* group upon the following: (1) The adults congregate upon fermenting food materials. (2) Periodic movement (walking) on the part of the individuals brings them into the immediate vicinity of other individuals, and then either chance physical encounter or visual ability brings two individuals into physical contact. (3) This is abetted by the fact that sexually potent males of this group normally tend to "investigate" all individuals that come within their range of vision.

## Typical Mating Behaviors

### *D. fumipennis*

A male, upon becoming aware of another individual within close proximity, approaches and taps the fly, then moves very slightly toward the rear of the

courted specimen. If another male has been tapped, he usually reacts by scissoring his wings and trying to tap the suitor with his fore legs. If a female has been approached, the male then circles to the front and faces her. He may be directly in front of her or diagonally located so that he faces one of her eyes. In this position, he extends one wing to 90° from his body and depresses it, at the same time curving his body, especially the abdomen, slightly toward the side of the extended wing. The tip of the abdomen is also a bit depressed. Then the fore leg on the same side as the extended wing is raised and straightened to its full length, forming almost a 40° angle with the horizontal plane. The rigidly extended leg is slowly and deliberately depressed and moved from side to side until it touches the female's body. This posturing before the female may last from less than one to several seconds. The male then circles to the rear of the female, dropping the wing into normal position as he runs. The female may spread while the male is posturing in front of her, or while he is circling, but usually she does not and the male comes to a halt directly behind her. If she ignores him, he will usually lick her whereupon she kicks with her hind legs and may decamp. If she extrudes violently, courtship may cease. Normally in the case of decamping and usually even when extruding occurs, the male will then circle to the front and posture again. As long as he retains contact with the female, the tapping is not repeated and the male may go through a whole series of posturing, circling to rear, circling to front, and posturing again. When the female accepts, she spreads her wings about 45° from the sagittal plane, droops the abdomen very slightly, and holds this position until the male licks, mounts, and inserts the phallus all in one motion. The female then relaxes her wings, letting them rest against the sides of the male, and she slightly contracts her abdomen. The male mounts moderately high and usually grasps the apex of the first section of the costa with his fore claws. Before grasping with his fore legs, he often may extend them straight forward and upward, hold them in this position for at least one second, and then lower them to grasp the female's wings. As soon as copula is accomplished, the male strokes for several seconds the area of genitalic union with his hind legs. The pair is then quiet until shortly (15 to 60 seconds) before the end of the copulation, when the female commences to kick with her hind legs and switch her abdomen laterally. The male dismounts by turning 180° and withdrawing the phallus. Unlike other species of the group, he will not immediately again court a female.

*Variations.*—The male may cease courtship at any stage except during posturing in front of the female and circling to the rear. The female can break off courtship at any time by decamping. Often a non-receptive individual will extrude, raise her abdomen and curve it toward the male as soon as he approaches and taps her. Receptive females may spread while the male is posturing or while he is sitting at the rear.

Often two males will court the same female. Bedlam then ensues; the males omit the posturing and go directly from tapping to the rear. The male who succeeds in getting behind the female aggressively licks her and the second male usually places himself behind the first male and licks. No female has been observed to accept a male when two males were courting her simultaneously.

In posturing, the fore leg stretching may be omitted, but, if utilized, some part of the courted individual is always touched by the leg.

### *D. nebulosa*

Both sexes of this very active, nervous species have the habit of lifting, spreading, and then dropping the wings back into normal position. At the same time, the entire abdomen is raised and dropped as the wings come back into place. This

motion appears to enable the individuals to become aware of the presence of another fly at a relatively great distance. Actually the other species of the group have similar motions, but the extent is so small that it is not noticeable to the naked eye.

The male approaches the side of the female, taps, upper-cuts, and then circles to the front, faces the female and scissors one wing. At the same time he curves the abdomen toward the same side as the scissoring wing and extrudes from the anus a small grey sphere of fluid. Normally he postures thus in front of the female until she spreads her wings or decamps. If she does not spread he may circle to the rear, then to the front again, and repeat the posturing. When the female extrudes she rarely elevates the body and never more than 20°. If she extrudes vigorously and his head is close to her genitalia, he turns and runs away, dropping his abdomen and clawing at his face. While he is in front of her, if she tries to walk forward and avoid him, he skittles from side to side as if to intercept and stop her. As soon as she spreads her wings, he circles rapidly to the rear, licks, mounts, and inserts the phallus in one movement.

She spreads the wings from 45° to 90° from the longitudinal axis of her body. He mounts well forward, grasps the middle of the costal margin with his fore leg and then her wings are rotated and folded backward so that each wing stands on edge with its surface parallel to the sagittal plane of her body and with the costal margin upward. After a second, the wing rotates into normal position and her abdomen, which has been contracted after he mounted and while the wing was on edge, now elongates to approximately normal position and he slides backward as a result of these combined movements. He strokes the area of genitalic union for a few seconds and then both individuals are quiet until 15 to 30 seconds before the termination of copulation. The female begins to kick with her hind legs, walks about and switches laterally her entire body. These motions cause the male to lose his fore tarsal hold and he slides back almost onto his back. The male turns 180° to dismount. He will almost immediately court and copulate with other females; he may court the female with whom he has just copulated but she always rebuffs him.

*Variations.*—When a male courts another male, the courted individual brusquely flicks his wings and jumps about so that he faces the suitor. Occasionally they will then scissor their wings.

In courting the female, scissoring by the male may occasionally occur when he is behind the female.

### *D. capricornis*

The male, aware of the proximity of another individual, approaches the side and taps. If the courted fly is another male, he spins and faces the suitor, scissors his wings, taps with his fore legs, and then both separate. If a female has been approached, she usually spars; the male circles to the rear and stops close behind the female. He then extends one wing 90° and vibrates and at the same time extends the middle leg of the same side toward the female and it also is vibrated. These movements are accompanied by a slight curving of the body and depression of the tip of the abdomen, which results in the male seeming to crouch slightly. Vibrating occurs as periodic bursts of movement interspersed between other activities which in turn are somewhat controlled by the female's responses. When the male goes to the rear, the female often extrudes and elevates her abdomen, and then the male usually upper-cuts although he sometimes vibrates. If she ignores him, he will vibrate and then lick her genitalia. She then kicks and may decamp. In any case, if she is non-receptive the male, after a short period, will circle either to the side or still further toward the front so that he faces the female's eye and starts over again. The pattern of side to rear to

side to rear may be repeated numerous times before the male tires or the female accepts. When the female accepts, she spreads her wings, usually about 45° from the median, and the male licks, mounts, and inserts the phallus. He mounts moderately high and typically holds on to the second section of the costa. Both sexes kick with their hind legs during the first two minutes of copulation, but the male particularly rubs the region of genitalic union. The female's abdomen which dropped slightly when she spread is usually strongly sucked in. Females are often restless during the entire copulatory period, but invariably one to five minutes before termination the female begins to switch her abdomen, kick and walk about. All this causes the male to lose his fore tarsal hold and to slide backwards. From time to time he may partially regain his original position, and he invariably determines the termination of copulation by turning 180° to dismount.

*Variations.*—The mid leg is not always vibrated at the same time as the wing. Occasionally the males stamp when they are rebuffed. Unlike what happens in the other species, males have been observed several times trying to mount without the female spreading and in one such case the individual was successful. There is always the possibility, although it was not observed, that this particular female spread her genitalia even though she did not spread her wings. The females do not decamp as readily as do those of other species, but they can elevate the abdomen greatly when they extrude. The males will court all and copulate with receptive females immediately after the completion of a copulation.

### *D. sucinea*

The male approaches the side of the female, taps her, and circles to the rear where he vibrates one wing. If she extrudes and elevates her abdomen, he will upper-cut her abdomen and wings, but if she just extrudes or ignores him then, after or during a period of vibration, he will lick her genitalia. She then kicks and usually decamps. He may pursue her or may circle to the front to stop her, then again to the rear to vibrate. As he races to the rear, he may droop one wing slightly. When the female spreads, he licks, mounts and inserts the phallus, assuming a relatively high position. The female's abdomen is greatly shortened due to contraction, and the male normally grasps the first costal section. The male strokes the area of genitalic union, the female usually remaining quiet although she may both kick and shake. At approximately the end of the first minute, the female forcibly elongates the abdomen. At the same time she may kick with her hind legs or clean the dorsal surface of her wings. The net result always is that the male is displaced backwards and may completely lose his first tarsal grasp. The pair then remains relatively quiet until three to five minutes before the end of copulation when the female again begins to kick, then violently to switch her abdomen interspersed with walking about. Finally the male turns 180° and dismounts. He is immediately ready to court and copulate again.

*Variations.*—There seems to be a relatively small amount of variation in the behavior of this species. The female decamps more often than does that of *capricornis;* the male will stamp and often lick the female if she does not spread. The female is often restless during copulation, but this may be partly because of the long copulatory period.

### *D. willistoni*

Males of this species approach the side of another individual, tap and then upper-cut. If another male has been courted, he reacts by wing scissoring, spinning about and perhaps even fighting with the suitor. Such fights involve butting, stamping, and use of the proboscis. If a female has been accosted, then the male circles to the rear and, taking up a posi-

tion behind the female similar to that assumed by *sucinea* and *capricornis*, he then proceeds to vibrate one wing periodically; he may also lick and upper-cut the female as well as stamp both of the fore legs. These actions do not occur while he is actually vibrating but in the time between periods of wing motion. While vibrating, legs of the opposite side from the moving wing are depressed and those of the same side are elevated, and thus the longitudinal axis of the body is slightly rotated. At the same time, the tip of the abdomen is curled slightly downward and the insect appears to be crouching. While the wings are still folded over the abdomen and before vibration starts, they tremble, i.e., there are tiny vertical motions accompanied by slight scissorings of the wings. Sometimes the uppermost or top wing may be snapped up and down very rapidly, reaching almost a vertical position on the upmovement. These trembling motions also occur when the male first becomes aware of an individual in his near vicinity. If, after a short time, the female does not accept him, he will circle her completely and return to the rear position. This circling is not often observed with virgin females because of their high receptivity. The vibration is different from that of *sucinea* and *capricornis* in that it is a fluttering type of vibration rather than a buzzing form. The female may extrude, although she does not do so as violently as do *capricornis* and *fumipennis;* usually she rebuffs the male by decamping, in which case he usually follows unless she flies. When receptive, the female spreads her wings to full 90° from the median and slightly droops the abdomen and extends the genitalia. The male licks, mounts and inserts the phallus, at which time the female contracts the abdomen and usually folds her wings. Often the female will hold the wing extended for one to two seconds after the male has mounted, and then the male has difficulty in holding onto the abdomen. The male mounts high and as soon as the female's wings are folded back, he grasps the area of the first section of the costal. The female immediately begins to kick and stroke the area of genitalic union with her hind legs, and the male may react by kicking at her hind legs with his hind legs. The female also may switch, but then the pair become quiescent and remain so until shortly before the end of copulation. The female begins to push vigorously against the male's abdomen with her hind legs, specifically the apices of the tibiae. This causes the male to lose his fore tarsal grasp and he slips backwards. He determines the time of breaking when he turns 180° and dismounts. He is then ready to court and copulate again.

*Variations.*—The male, especially if he is sexually excited, may not approach the side but will come directly behind the female with his head under her wings. Although difficult to determine, he probably always upper-cuts before he vibrates. Like all other species, the amount of extrusion by the females is variable but certainly the maximum is less than in any of the other species.

### *D. equinoxialis*

This species is similar to *willistoni* in every respect except as follows: (1) The individuals are lethargic while *willistoni* is equalled in activity only by *nebulosa*. *D. equinoxialis* spends most of its time quietly sitting with short bursts of activity periodically interrupting the quietness. (2) The females are extremely nonreceptive which results in a great deal of circling when the male is courting the female. (3) When the female does accept the male, she spreads her wings only about 45°, elongates and droops the abdomen excessively and spreads the genitalia greatly. (4) The male does not mount as high as does that of *willistoni*. (5) As the male inserts the phallus, the female slightly contracts her abdomen, but during the first minute of copula she extends the abdomen and depresses its tip, thus pulling the male back-

ward and also elongating his abdomen. (6) Several minutes before the termination of copulation, the female begins to switch as well as kick. Thus the male acts exactly like *willistoni,* but the female acts somewhat like *sucinea.*

*Variations.*—These are incompletely known, due to the small number of observations. Some females act similarly to *willistoni* in keeping their wings spread for 30 to 60 seconds after the male has mounted. One female observed did not elongate the abdomen excessively but the male that was involved in this copula was displaced backwards as she closed her wings.

## Discussion

The mating behaviors of the six species can be divided into six phases for analysis: (1) Initiation; (2) posturing; (3) restimulation; (4) mounting; (5) insemination; (6) dismounting. Each of these phases consists of certain actions and reactions between the individuals. Each reaction, of course, calls forth a further action. Under certain conditions these follow one upon the other until mating is successfully completed. When receptive members of opposite sexes are involved in these activities, one is tempted to say that the courting individuals can "recognize" each other. Actually there is no reason to introduce such a complicated concept because a simple and more lucid thesis is that each action elicits certain stimuli which in turn determine what the reaction shall be. Thus, when a male taps another fly, he receives as a result of his action certain stimuli which result either in courtship being broken off or in its being continued. Likewise, the tapped individual receives stimuli which elicit a response, the type depending upon the condition of the tapped individual as well as the tapper or suitor. Observations both on single pairs of individuals and on mass cultures indicate that the majority of courtships that are initiated do not terminate in successful mating. These unsuccessful courtships are broken off at some point usually during the first two phases, but sometimes during restimulation.

*Initiation.*—The male normally initiates courtship by approaching head-on the side of another individual, tapping and then upper-cutting (table I). If an individual of another species has been approached, the males of every species of this group or complex all break off courtship at this point. If a male of the same species has been tapped, then the courted male responds violently, usually spinning and tapping the suitor (the initiating male) and courtship breaks off shortly thereafter. When a female of the same species has been accosted, she responds by one of the following methods, i.e.: (1) She will indicate non-receptivity to his overture by extruding her genitalia and curving her abdomen toward him at the same time; she sometimes decamps, but this is rare (table I). (2) She shows no apparent response except perhaps to spar with her middle leg.

There are variations on the part of all individuals concerned in such initiating actions. Some males of *willistoni, capricornis, sucinea* and *equinoxialis,* especially those that are apparently sexually excited, do not approach the side of the courted individual but immediately go to the rear and then upper-cut. Under such conditions, the females react just as above. Normally the male initiates courtship but extremely receptive females of *nebulosa* will approach males, butt and tap them, thus starting the mating sequence. At the other extreme, highly non-receptive females of all species will extrude when any and all individuals come within their immediate vicinity and without any physical contact occurring between the individuals.

Having tapped a female of the same species, the male usually proceeds to the next phase of courtship regardless of the female's response. If the female extrudes violently, if she decamps or if the male himself is lethargic (low sexual urge for some reason), then courtship may be

terminated at this point. This occurs in a tiny minority of instances.

*Posturing.*—After initiation, the male immediately goes to the posturing position (table I). *D. nebulosa* and *fumipennis* go to the front, usually placing themselves directly before one of the female's eyes. *Nebulosa* postures by scissoring one wing, curving the abdomen toward the scissoring wing and extruding a small sphere of liquid from the anus. *Fumipennis* extends one wing to 90°, depresses it and at the same time stretches and elevates the fore leg of the same side, then depresses this leg and, by lateral movements if necessary, touches the fe-

TABLE I. *Mating behavior elements in the* Drosophila willistoni *group*

| Activity | *fumipennis* | *nebulosa* | *capricornis* | *sucinea* | *willistoni* | *aequinoxialis* | Phase when it may occur* |
|---|---|---|---|---|---|---|---|
| ♂ approaches side ♀ | yes | yes | yes | yes | yes | yes | 1 |
| ♂ taps side ♀ | yes | yes | yes | yes | yes | yes | 1 |
| ♂ upper-cutting | yes | yes | yes | yes | yes | yes | 1, 2 |
| Non-receptive ♀ responses: (a) Extruding | yes | yes | yes | yes | yes | yes | 1, 2, 3 |
| (b) Decamping | yes | yes | yes | yes | yes | yes | 1, 2, 3 |
| (c) Kicking | yes | yes | yes | yes | yes | yes | 2 |
| Position of ♂ posturing | front | front | rear | rear | rear | rear | — |
| Posturing movement: (a) Wing waving, fore leg searching | yes | no | no | no | no | no | — |
| (b) Wing scissoring and abdominal "sphere" | no | yes | no | no | no | no | — |
| (c) Wing vibration | no | no | yes | yes | yes | yes | — |
| (d) Mesothoracic leg vibration | no | no | yes | no | no | no | — |
| Restimulation activities: (a) Completely circles ♀ | no | no | no | yes | yes | yes | — |
| (b) Returns to posturing position | yes | yes | no | no | no | no | — |
| (c) Returns to side | no | no | yes | no | no | no | — |
| Receptive responses of ♀: (a) Spreading wings | yes | yes | yes | yes | yes | yes | 1, 2, 3 |
| (b) Elongating abdomen | yes | yes | yes | yes | yes | yes | 1, 2, 3 |
| (c) Spreading genitalia | yes | yes | yes | yes | yes | yes | 1, 2, 3 |
| ♂ licks ♀ when mounting | yes | yes | yes | yes | yes | yes | |
| ♂ grasps wing of ♀ when copulating | yes | yes | yes | yes | yes | yes | |

TABLE I. *Continued*

| Activity | *fumipennis* | *nebulosa* | *capricornis* | *sucinea* | *willistoni* | *equinoxialis* | Phase when it may occur* |
|---|---|---|---|---|---|---|---|
| ♀ activity during insemination: (a) Displaces ♂ backward by elongating abdomen | no | yes | no | yes | no | yes | |
| (b) Displaces ♂ backward by closing wings | no | yes | no | no | no | no | |
| (c) Strokes genital area immediately after ♂ mounts | no | no | yes | no | yes | yes | |
| ♂ activity during insemination: (a) Stroking genital area immediately after mounting | yes | yes | yes | yes | no | no | |
| (b) Retention of grasp of ♀ wings | yes | yes | yes | yes | yes | yes | |
| ♀ termination activity: (a) Kicking | yes | yes | yes | yes | yes | yes | Great variation in these activities. The male is solely responsible for termination of insemination. |
| (b) Switching | yes | yes | yes | yes | no | yes | |
| (c) Walking | no | yes | yes | no | no | no | |
| ♂ responsible for termination of copulation | yes | yes | yes | yes | yes | yes | |
| ♂ immediately ready to court females | no | yes | yes | yes | yes | yes | |
| Duration of copulation: Minimum | 4'0" | 1'10" | 6'30" | 15' 0" | 9' 0" | 14' 0" | |
| Maximum | 5'30" | 2'30" | 14' 0" | 35' 0" | 27' 0" | 19' 0" | |
| Mean | 4'20" | 1'37" | 9'55" | 20'33" | 14'41" | 15'55" | |
| Median | 4' 0" | 1'20" | 9'30" | 19'30" | 15' 0" | 15'15" | |

* 1—initiation; 2—posturing; 3—restimulation; 4—mounting; 5—insemination; 6—dismounting.

male. Having postured, the males of these species circle rapidly to the rear. The other four species go to the rear to posture. *Capricornis* intermittently vibrates one wing and the mesothoracic leg of the same side. *Sucinea, willistoni* and *equinoxialis* intermittedly vibrate one wing, but whereas *sucinea* and *capricornis* display a buzzing type of vibration, *equinoxialis* and *willistoni* show a fluttering type of vibration.

The females respond to the males' posturing in one of the following three ways: (1) Highly receptive females

spread the wings and genitalia which in turn causes the male immediately to copulate. (2) The female indicates non-receptivity by decamping or extruding and kicking. (3) The female shows no visible reaction and seemingly ignores the male.

*Restimulation.*—Only rarely is a female so receptive that she will accept the male after a single posturing. Almost invariably, the male in the rear position, after posturing, finds the female non-receptive. He then usually stamps his fore feet and attempts to lick her genitalia, although there is considerable variation both individually and between species. *D. sucinea* is very aggressive; *fumipennis* and *equinoxialis* are less so, and *capricornis* is least aggressive of all. If the female is non-receptive, the male restimulates her by a specific maneuver (table I). *D. fumipennis* and *nebulosa* males return to the front and posture again; *D. capricornis* circles toward the front, usually in front of her eye, and then returns to the rear to posture. All the other species completely circle the female, return to the rear and reposture. This may be repeated many, many times before the female accepts or the male quits. All males intersperse their unsuccessful courtships with periods of rest. Individual vitality apparently determines the length and number of the rest periods.

*Mounting.*—Eventually posturing and restimulation elicit one of the following responses from the female: (1) She becomes progressively less receptive, which she indicates by extruding her genitalia violently and lifting her abdomen high into the air or by decamping. (2) She indicates receptivity by spreading her wings, extending her abdomen and spreading the genitalia.

The non-receptive response finally causes the male to cease courting either because he breaks off the courtship voluntarily or because the female decamps and thus ruptures the proceedings. Usually the male will try to head off a decamping female by circling rapidly ahead of her but this is not always possible, particularly when she decamps by flying. If two or more males are simultaneously courting a non-receptive female, they may often lose contact with her when they start courting each other.

In general, the male does not attempt to mount until the female spreads her wings. Occasionally a male tries to mount before the female has spread her wings, but only once was a successful copulation observed under such conditions. Wing spreading is not by itself the critical or sole action that stimulates mounting (table I). It is merely a part of a complex action that also involves spreading of the genitalia and extension of the abdomen. These latter movements are small and hard to detect except in the case of *equinoxialis* and some individuals of *willistoni* where both are pronounced. During eleven consecutive hours of observation of two pairs of *equinoxialis*, the males courted the females over five hundred times, yet were stymied because the females did not spread, i.e., did not spread their wings and genitalia, and did not elongate and droop the abdomen. This also appears to be the reason why non-receptive females need not extrude or decamp in order to repel the males, but can accomplish that end apparently by merely ignoring them.

If the female responds to the male's courting overtures and indicates receptivity by spreading, then the male copulates at once. All six species do this by licking, mounting, and inserting the phallus as one complex, coordinated movement (table I). All males, except some individuals of *fumipennis*, grasp or attempt to grasp some part of the female's wing at the same time.

*D. fumipennis* males are able to mount and complete a copulation without going through the initiation and posturing phase of courtship, as shown by the following example. A male *fumipennis* posturing in front of a female elicited the spreading response from her but, before he could circle to the rear and mount, a

second male which happened to be passing just behind the female as she spread quickly accepted the invitation and mounted the female in a normal and typical manner. The first male, arriving a fraction of an instant after the mounting had been accomplished, was frantic and tried desperately but unsuccessfully to dislodge the interloper. The copulation was completed in five and one-half minutes and seemed normal in all respects. Probably males of the other species would react in a similar manner, but such a combination and the resultant reaction have been observed only in this one instance.

*Insemination.*—The first part of the insemination period involves activity that probably is concerned with the proper positioning of the genitalia (table I). Females of *D. willistoni* rub the area of genitalic union with their hind legs. In doing so, they strike the male's abdomen and he responds by leg movements to fend off the female. Apparently *equinoxialis* and *capricornis* follow a similar pattern. In the other three species, the males rub the region of the genitalia with their hind legs while the female is quiet except that some females of all species switch their abdomens at this stage. At the same time or shortly thereafter, the females of *sucinea* and *equinoxialis* extend the abdomen, a movement which drags the male backwards from the well forward position he assumed on mounting. *D. nebulosa* accomplishes the same end by the manner in which the female closes her outspread wings and extends the abdomen. *D. capricornis, fumipennis* and *willistoni* show no such behavior although, if the female switches just after the male has mounted, he may be dislodged backwards and temporarily lose his fore tarsal grasp. A period of relative quiet then ensues, punctured in some pairs by occasional walking, shaking and kicking on the part of the female. Other males are normally attracted to the pair, and if they pester the female she will raise her abdomen, lifting the copulating male, and partially extrude. Shortly before the male dismounts, females normally commence activity that appears to be an attempt to dislodge the male. The *D. willistoni* female applies the ends of her hind tibiae to the male's abdomen and pushes vigorously. Females of the five other species switch their abdomens, kick with their hind legs, and walk.

Table II shows the duration of copulation in 54 observed cases. The only other records of copulation duration for these species are given by Sturtevant (2). He lists one pair of *D. nebulosa* in copula for two minutes and one pair of *D. willistoni* in copula for 27 minutes. These records fall into the ranges that I have observed for these species. There is considerable variation in time both interspecifically and intraspecifically (see table I). In ascending order of duration, the range of *nebulosa* does not overlap that of *fumipennis*, and that of *fumipennis* does not overlap the range of *capricornis*. The range of *capricornis* overlaps that of *willistoni* which in turn overlaps that of *equinoxialis* and *sucinea*, but the latter one does not have a range that overlaps that of *capricornis*. *D. equinoxialis* is a rather frustrating species since 50 hours

TABLE II. *Duration of copulation in the* Drosophila willistoni *group*

| | |
|---|---|
| *fumipennis* | (4) 4′0″, (1) 4′30″, (1) 5′30″. Aver. 4′20″. Med. 4′00″. |
| *nebulosa* | (1) 1′10″, (2) 1′15″, (3) 1′30″, (2) 1′20″, (1) 1′55″, (2) 2′0″, (1) 2′10″, (1) 2′30″. Aver. 1′37″. Med. 1′20″. |
| *capricornis* | (1) 6′30″, (2) 9′30″, (1) 11′0″, (1) 14′0″. Aver. 9′55″. Med. 9′30″. |
| *sucinea* | (1) 15′0″, (1) 15′30″, (2) 16′0″, (1) 19′0″, (1) 20′0″, (1) 20′30″, (1) 22′30″, (1) 26′0″, (1) 35′0″. Aver. 20′33″. Med. 19′30″. |
| *willistoni* | (1) 9′0″, (2) 11′0″, (1) 12′0″, (2) 12′30″, (1) 13′0″, (1) 14′0″, (2) 16′0″, (1) 16′30″, (1) 17′0″, (1) 17′30″, (1) 22′30″, (1) 23′0″, (1) 27′0″. Aver. 14′41″. Med. 15′0″. |
| *equinoxialis* | (1) 14′0″, (1) 15′0″, (1) 15′30″, (1) 19′0″. Aver. 15′55″. Med. 15′15″. |

of observations, varying from a minimum observation period of 40 minutes to a maximum of 11 hours, were necessary to observe the four copulations listed in table II. In the case of every species other than *willistoni*, the median time is less than the average time. These data also parallel the morphological data in indicating that *sucinea*, *equinoxialis*, and *willistoni* are all very closely related and that *capricornis* is intermediate between *willistoni* and *fumipennis*, while, finally, both *fumipennis* and *nebulosa* are quite distinct.

There seems to be some evidence that the male is responsible for the intraspecific variation in copulatory time. Thus, a male of *nebulosa* that completed copulations with three virgin females in six consecutive minutes required 1′, 1′30″ and 1′20″ in that order. Two hours later when introduced to other virgin females, he copulated for 1′20″ and then ceased courting. These times are shorter than the average of 1′37″ for this species, but perhaps this is due to the high ambient temperature of 29° C. in which the matings were conducted. A *sucinea* male completed copulations with three virgin females in 55 minutes and required 15′ 30″, 15′0″ and 16′0″ respectively. The ambient temperature was 26° C. and the duration is considerably below that of the 20′33″ average of the species. However, another *sucinea* male completed copulations with two virgin females in 41 minutes, utilizing 20 and 19 minutes, respectively, almost average duration, but with an ambient temperature of 29° C. Apparently each male is relatively constant in the amount of time he needs to complete copulation with sundry females as long as the environmental conditions remain constant.

*Dismounting.*—The male of all six species determines the time of dismounting, regardless of the female's activity (table I). He may be almost on his back, supported mainly by his outspread wings as a result of her movements and yet he will not cease copulation. When he breaks, he turns either to the right or left 180° and withdraws the phallus. Both individuals then clean themselves, especially the genital area. All males except those of *fumipennis* are then again ready to court any female and copulate with those that are receptive.

The various elements of the entire mating behavior have been summarized in table I. While such a table can not possibly include all of the variations of the elements involved, it does give a condensed picture of the various actions and responses and indicates the behavioral differences that exist between the species. As can be seen from the table, the analysis of the six phases, and the descriptions of the courtships, specific behavioral differences exist between all of the species. These differences are least between such morphologically closely related species as *equinoxialis* and *willistoni*, and greatest between more morphologically diverse pairs such as *willistoni* and *fumipennis*. These differences are both qualitative, e.g., the position asumed by the male for posturing and quantitative, e.g., the extension and spreading of the genitalia. When compared with the morphological differences that exist between the species, it is to be noted that the behavioral differences are roughly quantitatively parallel to the morphological differences. This indicates that within this group behavioral differentiation has evolved at about the same rate as the morphological differentiation and that both have continued to further and further separate the various species. In the case of *willistoni* and *equinoxialis*, the behavioral differences are greater than the known visible morphological differences which perhaps indicates that behavioral differentiation precedes morphological differentiation in the time sequence.

## Summary

Initiation of courtship, mounting, and the termination of copulation are similar in all six species of the *willistoni* group. Specific qualitative differences exist be-

tween each of the species and these are restricted to posturing behaviors of the male, the female activity of signifying acceptance of the male, and the actual insemination period. These differences roughly parallel the morphological differences that exist between the species except for *willistoni* and *equinoxialis* between which the behavioral differences are greater. Thus the evolution of the sexual behavior has approximated quantitatively the evolution of the morphological characters.

Courtship may be broken off at any point betwen the male's approach of the female and the actual copulation. Acceptance or non-acceptance in the intraspecific courtships of this group is a function of the female and she can rebuff the male by various means but especially by extrusion of the genitalia. Unless she is receptive, the male is unable to copulate.

Preliminary evidence indicates that the male will break off courtship at the first phase, specifically the tapping action, if the female approached belongs to another species.

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# OBSERVATIONS ON EVOLUTIONARY RATES IN HYPSODONTY

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An examination of fossil horse teeth from the American Cenozoic faunas reveals a gradual increase in hypsodonty (the height of the crown of the cheek-teeth) from late Lower Miocene to Pleistocene time. This is illustrated in the equine (*Merychippus, Calippus, Pliohippus, Equus*) and hipparion (*Merychippus, Hipparion, Neohipparion, Nannippus*) groups of hypsodont horses. There is some increase in crown height in the other groups of horses but it is only slight or is associated with an increase in the dimensions of the tooth as a whole. Hypsodonty is displayed also in other families of North American plains animals (Castoridae, Heteromyidae, Geomyidae, Mylagaulidae, Rhinocerotidae, Camelidae, Antilocapridae, etc.) at that time, but for the most part it is less accentuated and not as well documented as in the Equidae. In this report attention is directed to a combination of phenomena closely associated with this evolutionary progression in the horses.

Simpson (1944, p. 16) states that—"hypsodonty is an important unit character on the criterion of selection value, but morphologically it is the resultant of two other characters which are correlated in one way and uncorrelated in another, and genetically it is undoubtedly controlled by at least two and probably many genes that simultaneously control other, quite distinct phenotypic characters." In this connection it may be useful to enumerate some of the features in the skulls and dentitions of Cenozoic horses that seem to have direct bearing on the inception and evolution of hyposodonty in the Equinae. Development of the crochet and the hypostyle as employed in the closure of the pre- and postfossettes was essential in the formation of a basic pattern for an effective triturating surface. From this basic pattern, then, in certain Lower Miocene species of *Parahippus*, the teeth in a succession of species and genera evolved into structurally sound hypsodont teeth. An additional strengthening factor was the deposition of cement which filled the inflections and fossettes and even reinforced the outer surface of the tooth. Other important considerations in this evolutionary process would be the gene or genes controlling an increase in the depth of the facial and the mandibular regions to accommodate higher crowned teeth, and those controlling the deposition of enamel and dentine.

Origin and evolution in Tertiary mammals, particularly in the hypsodont horses, has usually been correlated with a change in diet. This dietary change probably involved a shift from browsing on softer herbaceous plants to grazing on harsh or siliceous grasses which acted as abrasive agents on the teeth. Only a few authors have mentioned sand mixed in the food of the grazers as an additional source of abrasive material. These changes in turn, are associated directly with increasing aridity on the Great Plains of the middle and late Tertiary. It is observed that the appearance of widespread sandy deposits, together with the appearance of bunch grass (*Stipidium*,[1] Elias, 1935), and increasing aridity, are contemporary with the development of hypsodonty. M.

[1] Referred to the living genus *Stipa*—spear grass by some botanists.

K. Elias (1935, p. 25) in his very useful paper, "Tertiary Grasses and other Prairie Vegetation from High Plains of North America," cites literature and supports the evidence for late Cretaceous and Cenozoic grasses. To quote: "Only the presence of *Arundo, Phragmites* and perhaps *Bambusa* in the late Cretaceous and Tertiary beds was considered as being established on sufficient fossil evidence. The living representatives of these grasses are tall water-loving species which dwell along the shores of ponds and streams. The association of their fossil relatives with the arboraceous vegetation permits no doubt that in the late Cretaceous and Tertiary times they grew in the same environment." It is difficult to believe that shorter grasses also were not in these associations as they are in similar environments today. If siliceous grasses were present in the early Cenozoic and their presence affected the teeth of those animals that fed upon them why did not hypsodonty arise in several groups of mammals at that time? As a matter of fact, there is very little if any relative increase in the height of crown in the Eocene and Oligocene horses. It is impossible, however, to prove that these animals were not browsers exclusively. In this event it might be assumed that the siliceous grasses would have no effect on their teeth, though the grasses may have been present. On the other hand is there any reason to conclude that they fed only on other kinds of plants? I think we can assume, with reason, that the grasses were not as dry nor as harsh in the early Tertiary as they were in the Miocene and Pliocene.

A review of the literature reveals that there was a predominance of shales and sandstones from the Puerco, Torrejon and Tiffany in New Mexico (Granger, 1917); and in beds of the same age in Wyoming, Jepsen (1930) reports mostly sombre clays, and sandstones. The Eocene (see Van Houten, 1945), though containing much volcanic detritus, also shows considerable sandstone, clay and shale. Primarily clays occur in the Oligocene with intermittent deposition of sandy stream channels. Toward the close of the epoch a greater percentage of sandy deposits accumulated in the Great Plains area.

This continued in the Lower Miocene with the widespread deposition of sands and sandy silt in the Arikareean formations of Nebraska, South Dakota, Wyoming and Colorado. These formations must have been much more widely distributed, but subsequent erosion and deposition either removed or covered most of the deposits. Sand grains in the later Miocene and Pliocene average larger and these sandy flood plain and stream channel deposits predominate particularly in this region where they form most of the later Tertiary blanket.

Toward the close of the Arikareean the first records of bunch-grass appear. Elias discovered the siliceous husks of a small *Stipidium* in the late Lower Miocene Harrison beds of western Nebraska. This appears to be the first appearance of these grasses in the American Cenozoic.

Contemporary with these Harrison grasses we note the formation of cement on the teeth and a tendency toward heightening of the crown in some species of *Parahippus* in the late Lower Miocene. In these horses, is seen the basic dental structure for the development and evolutionary progression in the cheek-teeth of successive races of hypsodont horses from the Lower Miocene to the Pleistocene. Certainly there is not a better example of evolution in Cenozoic Mammalia and it is questioned whether a better succession occurs in any other group of organisms (figures 1–2).

The Lower Miocene evidently marked the beginning of plains conditions with a gradual change through moist to dry temperate climates in the later Pliocene. That these plains were exactly like the prairies of the Great Plains region as we know it today is quite unlikely, but they were considerably different from those in Eo-

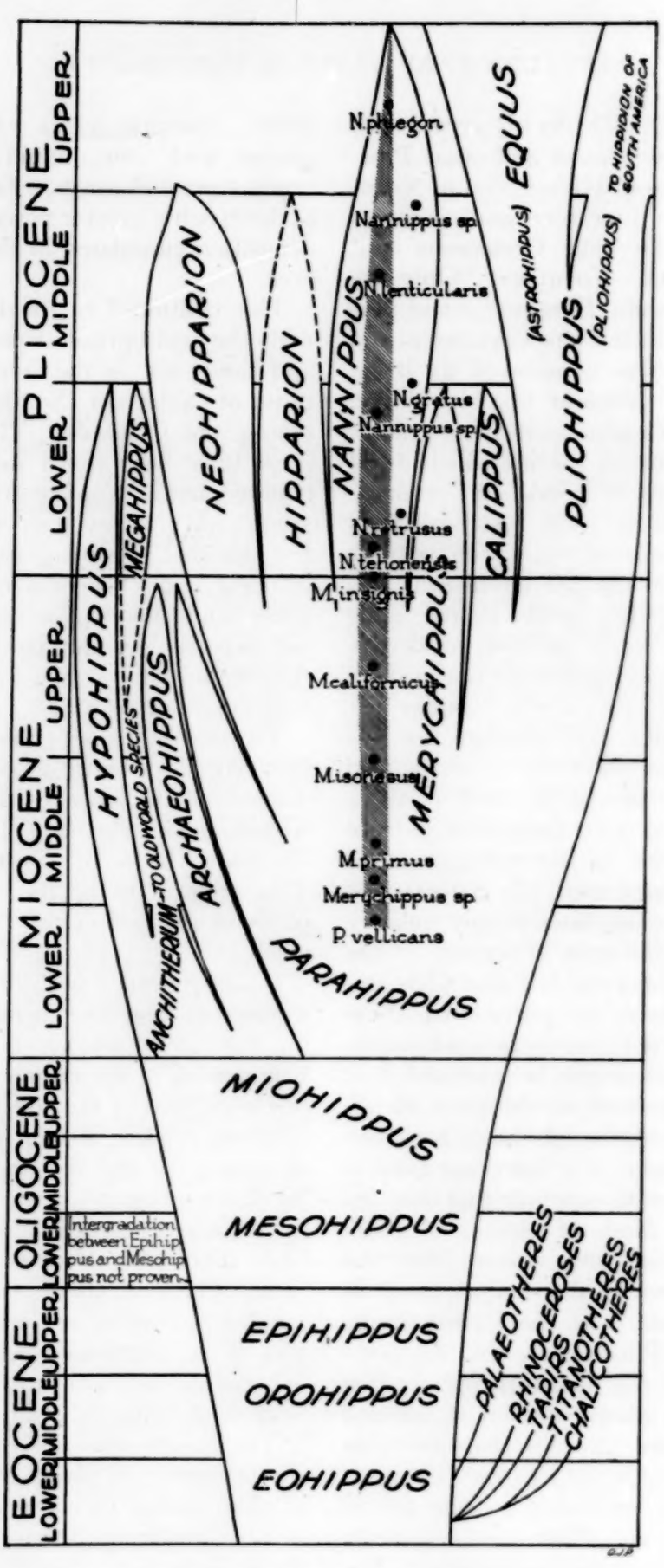


FIG. 1. A phylogeny of North American Equidae illustrating phyletic position of the *Parahippus vellicans* (Hay) to *Nannippus phlegon* (Hay) line of descent.

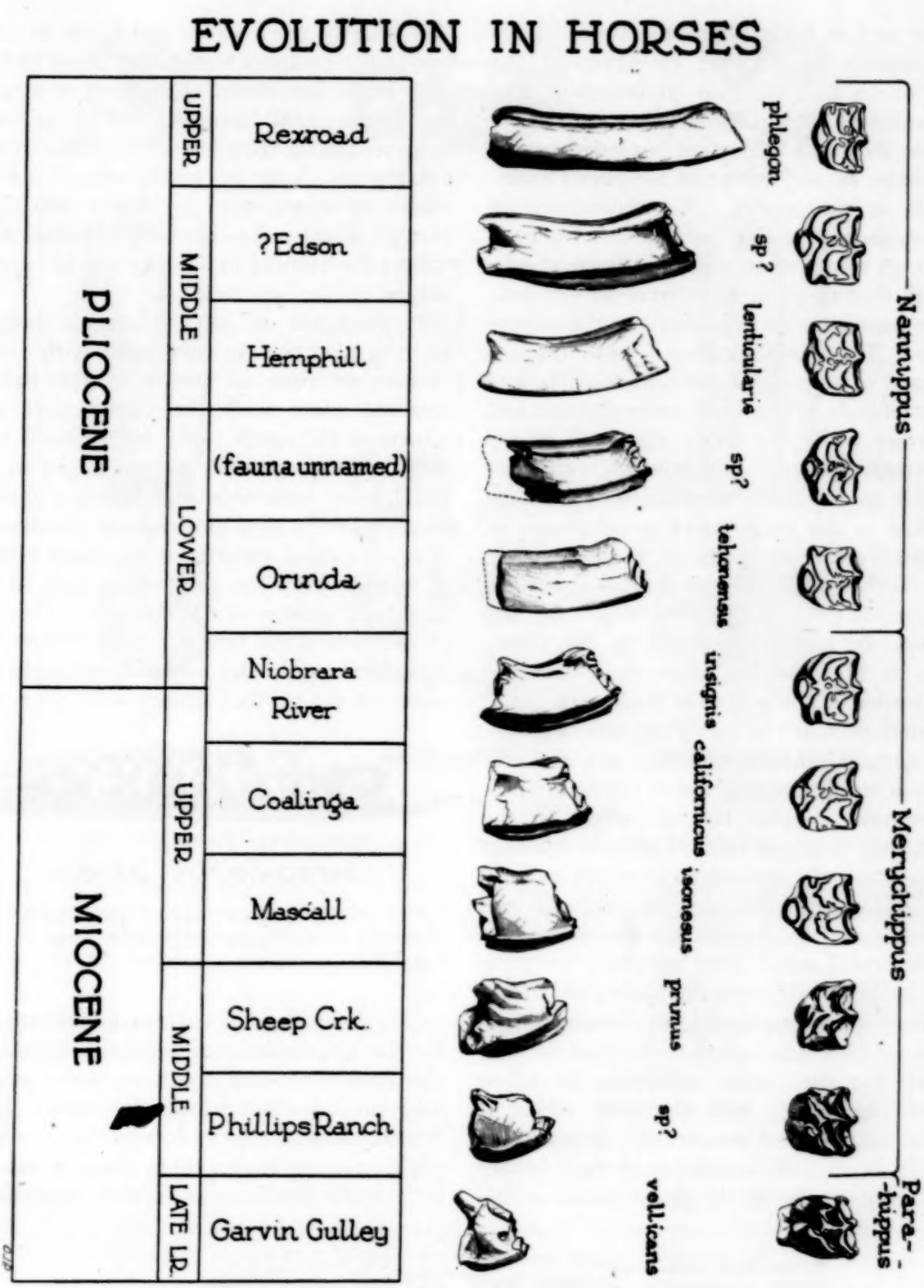


FIG. 2. Progressive hypsodonty as illustrated by unworn or slightly worn isolated horse cheek-teeth. The *Nannippus* sp. in the unnamed fauna from California is not well known and it might be questioned as to the position of this tooth in the late Clarendonian stage. Some of our research may clarify this problem in the next few years. The *Nannippus* sp. from the ? Edson fauna apparently is not in the direct line leading from *N. lenticularis* (Cope) to *N. phlegon* (Hay), but it does represent the stage of hypsodonty intermediate between those species. *N. retrusus* (Cope) and *N. gratus* (Leidy) are on a side line that evidently did not extend into Middle Pliocene time.

cene and in early Oligocene time. Thus apparently by another elevation of the region to the west, an environment was established in the Great Plains that became the most important recorded center of dispersal and evolution for plains mammals in the world. A combination of phenomena existing in this environment was so effective in wearing down cheek-teeth that there seem to have arisen definite protective modifications in the organism. These modifications were the increase in activity of the odontoblasts and ameloblasts to lay down more dentine and enamel while the tooth was still in the formative stage. Just how such changes arose as heritable modifications and resulted in the progressive development of higher crowned teeth or hypsodonty is certainly not clear from the fossil record.

Bunch grasses, possibly both long and short, became widespread in the Great Plains area shortly after their first appearance. The siliceous husks are found abundantly in the sandy stream deposits. A certain amount of silica and the dry harsh condition of grass in the later summer and autumn had its effect in the wearing down of teeth, but it is felt that this was only secondary in effect to sand as a foreign substance mixed with the grass. In this region a close inspection will reveal much sand adhering to grass or lodged in between the blades and stems where it was splashed after thunder showers or blown by winds. Thus it is possible for these sand grains to be taken into the mouth with the food where it acts as an effective abrasive agent.

It is a well known fact that horses grazing on the sandy plains pastures display heavily worn teeth, and those in the less sandy pastures to the east show teeth with much less wear. Ranchers in western Kansas and Nebraska tell of serious sickness among their horses resulting from grazing too long in sandy wheat fields where so much sand is blown into the young wheat. Local veterinarians attribute the ailment to too much sand being lodged in the stomach.

Hypsodonty in later Cenozoic horse teeth is also closely correlated with root closure or time of fusion of the basal dentinal plate with the upper part or crown of the tooth. My information on this is not as complete as one might wish but I have been able to observe a slight retardation in root closure and continuation of vertical growth in the cheek-teeth of species from the succeeding ages from Middle Miocene to Pleistocene. This is shown when the time of root closure is compared with the amount of occlusal wear on the tooth (figures 3–7).

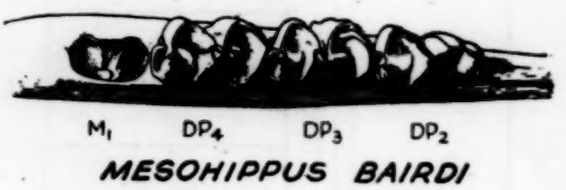


FIG. 3. Deciduous lower cheek-teeth of *Mesohippus* displaying only slight wear at the time $M_1$ is erupting.

The formation of enamel, as laid down by the ectodermal ameloblasts, begins at the crown of each tooth, spreads down over the sides and into the fossettes. The first centers of enamel formation are over each cusp or high point; these coalesce as growth continues. At the same time

FIG. 4. Deciduous lower cheek-teeth of *Nannippus* displaying considerable wear at the time $M_1$ is erupting.

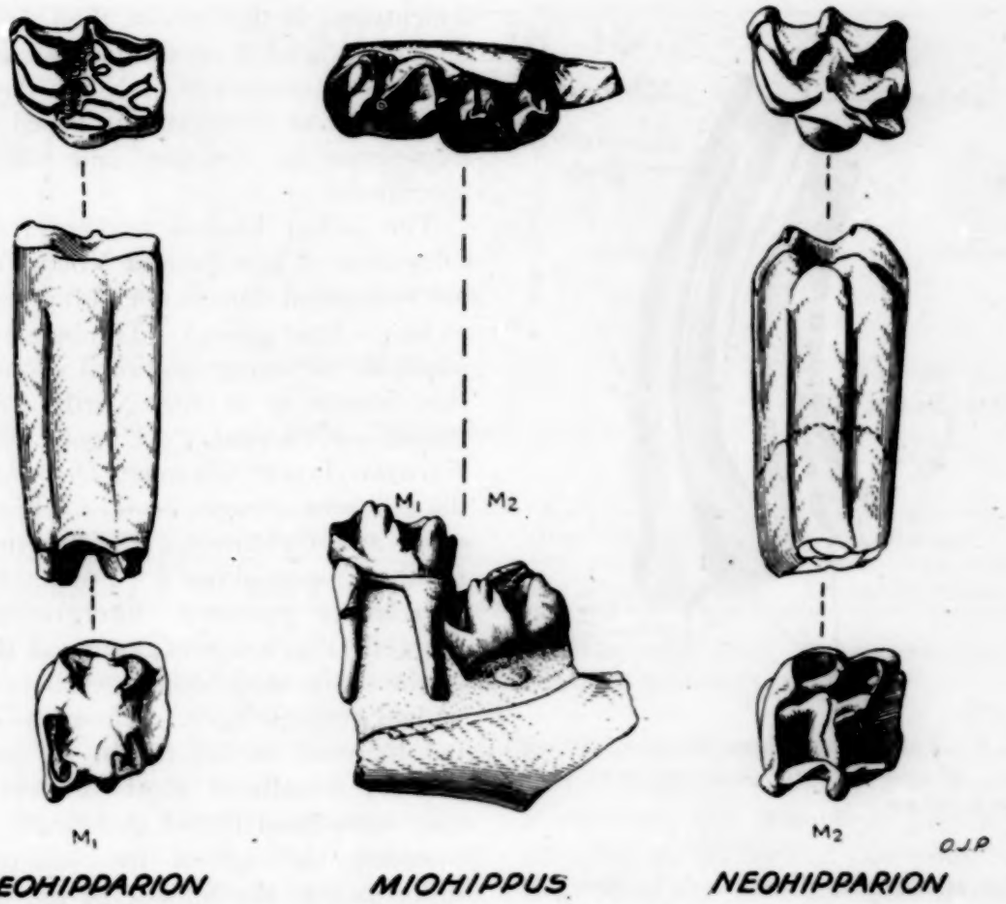


FIG. 5. FIG. 6. FIG. 7.

FIG. 5. *Neohipparion occidentale* (Leidy). $M_1$ slightly worn and showing basal dentinal plate fused with crown.

FIG. 6. *Miohippus* sp., John Day of Oregon, showing roots fused on $M_1$ before the enamel is worn through to expose dentine; the roots have not formed on $M_2$ as the tooth starts eruption.

FIG. 7. *Neohipparion occidentale* (Leidy). $M_2$ unworn and without basal dentinal plate, but bottoms of fossettes are closed.

in the dental papilla the mesodermal odontoblasts are forming dentine. The odontoblasts apparently persist throughout the life of the individual and continue to produce dentine so that the root canals become nearly, and in some individuals, completely obliterated. The full height of crown in a tooth is formed in *Miohippus* before the top of the crown erupts through the gums and the fusion of the roots to the crown is completed very soon after eruption, but in *Nannippus, Neohipparion* and *Pliohippus* the tooth not only erupts but displays some wear before the basal dentinal plate becomes fused with the crown and the height of crown is fixed. (See figures 5 and 7.) In *Equus* this fusion is more retarded than in the Lower and Middle Pliocene genera (figure 8).

The identification of species has been based on a multitude of characters in the cheek-teeth, i.e., crown height, shape of protocone, complication or simplification of fossette borders, prominence of styles, occlusal dimensions, isolation of hypoconal groove, degree of connection of protocone to protoselene, flatness of ectoloph, bifurcation of plicaballin, etc., not to mention similar characters in the mandibular teeth as well as other features in the skull and feet. It was found that a character constant in the identification of one species or group might be variable in another.

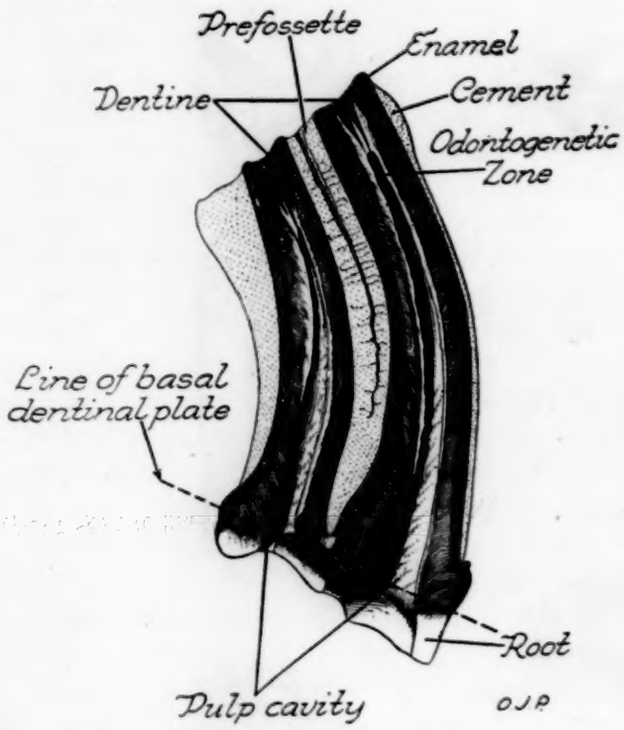


FIG. 8. *Nannippus retrusus* (Cope). $P^4$ with vertical section through paracone, prefossette, and protoselene.

The specimens illustrated in the phyletic sequence, *Parahippus—Merychippus —Nannippus,* are hand-picked but they represent my estimate of an average crown height for the species in successive faunas. It is true that there is much variation in crown height in these species, in some samples, even overlapping in range the species from preceding and the following ages. Though there are large series of isolated teeth from some faunal assemblages, it is difficult to find a tooth displaying the full crown height for a species. Teeth assume different proportions and different features in their occlusal patterns at different stages of wear, but a student familiar with the phyletic sequence in horses and also with first hand information on large series of teeth from given faunas can make an accurate estimation of crown height. Thus in identifying specimens these facts must be considered. For these reasons I have found it exceedingly difficult to compile satisfactory measurements on crown height in relation to coronal dimensions. The conclusions in this report, then, are based on my judgment of similarities and differences in specimens of North American late Cenozoic mammals as based on my experience in curating and identifying specimens.

The oldest known beavers from the Oligocene of Europe and North America are hypsodont though not as high crowned as in the later genera. The best evidence available on intergradational evolution in this family is in the North American genera—*Monosaulax* Upper Miocene, *Eucastor* Lower Pliocene, *Dipoides* Middle Pliocene, *Procastoroides* Upper Pliocene, and *Castoroides* Pleistocene (subfamily Castoroidinae). Fortunately for correlation purposes, intergradation of the generic characters occurs at the time of the designated boundaries between the named geologic ages. There was a gradual increase in the height of crown in the cheek-teeth of these beavers which may have been linked genetically with a breaking through of the fossettes and inflections to the outside of the tooth as well as a lengthening of lateral grooves on the sides of the teeth. Evolution of the tooth was relatively constant in the succeeding genera but there was an acceleration in hypsodonty in the late Pliocene and Pleistocene (figure 9).

Since there does not seem to be a continued acceleration between the Blancan and Pleistocene equivalent to that between the Hemphillian and Blancan time we may be confronted here with a distinct break in our faunal records at the close of the Middle Pliocene, which future collecting will clarify.[2] Therefore, though there is a distinct difference in the rate of evolution in hypsodonty with the oncoming of widespread glacial conditions it may not be as great as it appears from the evidence available.

The ecological conditions under which the castoroid beavers lived is not known, but a few inferences can be made. It is

[2] I have seen some evidence of faunas of this age from the Panhandle of Texas.

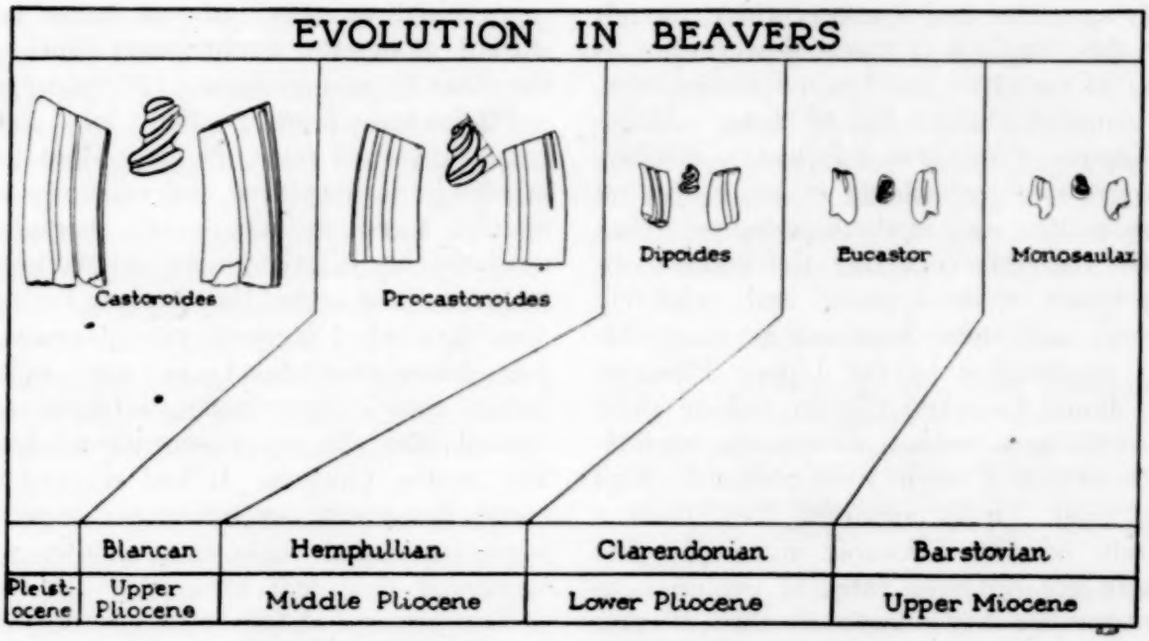


FIG. 9. Progressive hypsodonty as illustrated by slightly worn fourth lower prèmolars ($P_4$). The base of the *Procastoroides* tooth is semidiagrammatic.

likely they were water-loving animals since their remains are found so abundantly in stream channel deposits. The cause of their death in great numbers at any given time might be attributed in part to occasional severe droughts and disease. Possibly members of this subfamily were more like the muskrats in their habits and depended on green grasses and reeds for their livelihood. Thus in long periods of severe drought many of their special requirements would be eliminated, effecting heavy mortality. Scattered bones and teeth of the dead animals which had accumulated along the bank of dried up water courses could have been picked up by subsequent floods, then deposited in eddies or potholes in the stream. The ecology of these beavers, if this is an approach to a true picture of their environment and habits, is somewhat different from that of the contemporaneous nannippine horses, since the beavers were stream border and marsh dwellers, whereas the horses were open grassland types that frequented streams and waterholes when thirst impelled them. Nevertheless the same selective values may have operated in both phyletic lines. Abrasive sands would be as prevalent along stream borders and in adjacent swampy areas as in the open grassland. It is interesting to observe then, though hypsodonty was initiated earlier in the castoroid beavers than in the merychippine-nannippine horses, the rate of evolution was comparable in both lines; and in both there was an acceleration in the late Pliocene. Evolution in the cheek-teeth of American castoroidine beavers differed from that in horses in that the loss of roots in the Middle Pliocene *Dipoides* and in the subsequent genera in that line of descent did not occur in the horses.

The rate of hypsodont evolution in antilocaprids (*Merycodus-Antilocapra*), contemporaries of the beavers and horses, was slower but only slightly accelerated in the late Middle and Upper Pliocene. Hypsodonty in the camels with higher crowned teeth (*Paratylopus—Oxydactylus—Procamelus—Camelops*) is about intermediate in rate between that in the antilocaprids on the one hand and the horses on the other. But evolutionary rates in camels and antilocaprids cannot be accurately determined until the phyletic relationships of the genera in these families are better known. This is also true in the Heteromyidae, Geomyidae,

Mylagaulidae and Rhinocerotidae, though in the rhinos it is much slower than in any of the other families mentioned here.

Another phyletic line of horses (*Merychippus—Pliohippus—Equus*) displays progressive hypsodonty of an equivalent rate to that seen in the nannippine horses with the difference that the occlusal dimensions were actually and relatively larger and there was not as noticeable an acceleration in the Upper Pliocene. It should be noted that in making these observations occlusal dimensions in relation to crown height were constantly kept in mind. It is apparent then from a study of late Cenozoic mammals that there are different rates of evolution in progressive hypsodonty in the different orders, in the families of an order, in the genera of a family, and in the species of a genus. I have seen no evidence for macromutations.

Though populations of antilocaprids, of camels, of nannippine and other hypsodont horses in all probability occupied the same grassland areas it is not logical to assume that they occupied the same ecological niches in their environment. This together with certain physiological factors may account for the different rates of evolution displayed in given characters in different lines of descent. Even in the *Merychippus—Nannippus* line there is indication of distinct lines diverging from the main stem, in some of these (*N. retrusus* (Cope)—*N. gratus* (Leidy)) hypsodonty was still progressive but in others (*M. brevidontus* Bode) it showed no progression or was greatly retarded.

This non-progressive hypsodonty is further exemplified by *Merychippus* teeth described by J. T. Gregory (1942, pp. 225–228) in the Big Spring Canyon fauna.[3] The primitive merychippines represented by this Pliocene horse had started along the evolutionary lines of the other hypsodont horses (*Merychippus—Pliohippus—Equus*) but for some reason diverged from the hypsodont line in Hemingfordian time and evidently remained fixed in its dental characters until the late Clarendonian, when, in so far as we now know, they became extinct. Another kind of *Merychippus* (*Merychippus—Nannippus* line) in the Burge fauna, early Clarendonian, is more advanced than the more recent one from Big Spring Canyon. It had reached an early Barstovian evolutionary stage in hypsodonty and represents another unsuccessful divergent line.

The genus *Calippus* evolved to a degree where it became generically distinct from *Merychippus* in the late Miocene. The teeth were hypsodont but tended to decrease in size (*C. regulus* Johnston) and did not keep pace in progressive hypsodonty with *Nannippus* which also showed a decrease in occlusal dimensions. Present evidence indicates that it died out in the late Clarendonian or in early Hemphillian.

It should be noted that among contemporary horses the hypsodont ones are always more numerous. I assume that the hypsodont kinds occupied the open plains areas while the less progressive and lower crowned forms, at least in part, were ecologic competitors with anchitherine genera and possibly were semi-browsers.

Is there enough difference in the height of crown in the cheek-teeth of contemporaneous individuals in any given species of horse, or in succeeding generations of that species, or even in phyletic species from succeeding zones (i.e. stratigraphic zones of 1000–1,000,000 years duration, less than the time represented between any two successive faunas in figure 1) to make natural selection on the differ-

[3] Gregory considered these teeth as being close to *M. isonesus* (Cope) but I feel that they are even closer to *M. primus* (Osborn). This conclusion is based on the (a) attachment of protocone to protoselene, (b) crown height, (c) and dimensions of the teeth as a whole. There is no evidence that these teeth were secondarily deposited in the Big Spring Canyon sands and there is good reason to believe this horse was contemporary with that faunal assemblage.

ence in hypsodonty alone a determining factor in survival? I think not. After the group had continued to exist for perhaps three or four million years with the gene or genes involved in progressive hypsodonty linked with one or more genes controlling other factors of survival value, the degree of difference in hypsodonty in progressive forms could have become enough greater over the nonprogressive forms, provided other factors were equal, that the animals with higher crowned teeth would have distinct advantage.

If hypsodonty alone was a determining factor in survival it is difficult to explain why the *Hipparion* line became extinct at the close of the Clarendonian in America and the *Neohipparion* line failed to survive beyond the Hemphillian. These horses were as progressive in their hypsodonty as their contemporaries in the *Nannippus* and *Pliohippus—Equus* lines which continued into the Blancan on the same continent and in the same areas.

There are a multitude of factors, it seems to me, that might play a role as important as hypsodonty in survival or longevity such as hardness of dentine and enamel, mechanical (see Camp and Smith, 1942) and physical ability to secure food, to escape enemies, to produce and protect young, etc. All of these qualities must necessarily be related to a number of morphological and physiological variates, so many in fact, aside from their evaluation, that we cannot determine all of them even in living animals.

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# SECOND CHROMOSOME INVERSIONS IN WILD POPULATIONS OF *DROSOPHILA MELANOGASTER*

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## INTRODUCTION

Many authors have stressed the importance of chromosomal inversions as a possible mechanism of the primary stages of evolution in some if not all kinds of organisms. An inversion causes the transmission of a block of genes with extremely reduced probability of exchange of any of them by crossingover in an individual heterozygous for the inversion. This is to the advantage of the individual organism whenever such a block of genes is of selective value when acting as a unit.

The comparative genetics of *Drosophila* indicates that inversions have been by far the most common chromosomal change associated with speciation in this genus (Sturtevant and Novitski, 1941). Studies of gene sequences in present wild populations of the genus suggest that in some cases recent evolution has followed this path while in others alternative gene sequences do at least show different maximum rates of viability associated with seasonal environmental conditions. These observations are especially prominent in the work of Dobzhansky and his colleagues with *Drosophila pseudoobscura* and its relatives (Dobzhansky, 1944). Similar though less extensive observations have been reported existing in populations of *D. azteca* (Dobzhansky and Socolov, 1939), *D. algonquin* (Miller, 1939), *D. athabasca* (Novitski, 1946), *D. nebulosa* (Pavan, 1946), *D. funebris* (Dubinin and Tiniakov, 1946) and others.

The closely related species, *Drosophila melanogaster* and *simulans*, are differentiated by comparatively few inversions (Horton, 1939). In addition, fewer different inversions have been found in wild populations of these species than in populations of many of the above species. However, wild populations of *D. melanogaster* are far from being void of inversions (Dubinin, Sokolov and Tiniakov, 1937; Warters, 1944), and it is not unlikely that further evolution in this species will be associated with inversions. It is of interest, therefore, to determine the nature and distribution of chromosome inversions in wild populations of *D. melanogaster*. This paper is a report of the beginning of such a study on the wild population of *D. melanogaster* found in the commercial fruit orchards of South Amherst, Massachusetts, the study having developed from the work reported in Ives (1945).

## METHODS

The method of analysis followed in this study is similar to that described in Ives (1945). Wild flies were collected by means of half-pint bottles of fermenting fly culture medium or by hand-trapping. One second chromosome from each wild fly was first tested for its homozygous viability by means of a series of matings with the Bristle/Curly marker stock. It was then tested for the presence of an inversion by means of a crossover test with the 2-ple set of marker genes or a similar set of marker mutants, net black cinnabar brown proving the most satisfactory. When it was possible to do so, lethal-bearing inversion chromosomes were crosstested to determine if the lethal genes were identical. Finally, most of the

inversions were examined in salivary gland chromosome smears stained with aceto-orcein in order to determine their structural relations to each other. To facilitate this analysis the inversions were kept in stock balanced with net black cinnabar brown. The heterozygous larvae have normal colored malpighian tubes in contrast to the colorless ones of the homozygous cinnabar brown. The stock is continued in each generation by mating heterozygous males and homozygous net black cinnabar brown females. The salivary studies were made by my colleague, Dr. Taylor Hinton. I am very grateful to him for this important aid.

## Characteristics of the 1945 Collection

Studies have been made on flies collected from the local orchard area in 1938, 1941, and 1945. Details of the characteristics of the first two collections are found in Ives (1945). Inversion tests were made only on lines of the 1938 and 1945 collection. In order to understand fully the significance of the inversion data, the characteristics of the 1945 population must also be described.

Two successive seasons of severe frost damage to the fruit during the blossom stage, the first such losses in the 45-year history of the local commercial orchards, reduced fruit yield during both the 1944 and 1945 seasons to less than 5 per cent of the average annual yield for the previous ten years. Not only was the total crop reduced but the distribution of the fruit in the orchards was very irregular, large areas bearing no fruit at all and most of the apples being concentrated in widely separated areas of high ground which escaped frost damage. Also, because of the short crop, nearly all drop apples were harvested, whereas normally many poor quality drops are left under the trees where they become insect cultures.

It was easily observed that these unusual conditions reduced the maximum *D. melanogaster* populations of 1944 and 1945 to far below the numerical size of normal years. It was thought possible that they might also have a direct effect on the size of the minimum (hibernating) population in the winter of 1944–1945, and that this would be reflected in a change in the characteristics of the September 1945 collection when compared to the 1938 and 1941 collections.

Tests of 190 chromosomes of the 1945 collection showed that 87 (45.8 per cent) were lethal or semilethal, giving less than 17 per cent of non-Curly in the Curly × Curly test-cross. This is significantly lower than in 1941 and almost exactly the same as in 1938. Crosstests of 56 of the lethals gave 14 instances of identical lethals in the 1540 tests (0.91 per cent). This is more than twice the proportion of identical lethals in the 1938 population. (This test was not made on the 1941 collection.) A total of 45 different lethals were found in the crosstests. Of these 36 were found once, eight twice and one four times. This suggests a closer relationship of the lethals to each other than was the case in 1938, even though the difference in percentage of identical lethals in the 1938 and 1945 collections is not statistically significant. On the whole, however, there appears to have been very little diminution of the breeding population at its point of minimum size following the very poor apple year of 1944. At that point in its annual cycle the size of the breeding population appears to be largely independent of the size of the apple crop. Presumably, then, the flies do not hibernate as larvae in drop apples which escape freezing during the winter months.

The usual quota of visible mutations was extracted from the 1945 collection. Among them was the one which may be considered the type mutation for this population area, cardinal eye color. The best of the new mutations was an excellent recessive allele of the blackish eye color mutant, Henna, in the third chromosome. This allele, $Hn^{rl}$, has proved very useful in elementary class work.

## The Data on Inversions

The results of the analysis of the 1938 and 1945 collections for frequency of second chromosome inversions are given in the accompanying table. The class, lethals tested, includes both lethal and semi-lethal chromosomes. The class, non-lethals tested, includes all chromosome lines giving over 17 per cent of non-Curly in the Curly × Curly tests. Included in the 103 1945 lethals are 16 from an additional part of that collection which was not analysed sufficiently to be included in the data of the previous section. The data are somewhat different here than in the abstract of Ives and Hinton (1946) because in that summary semi-lethals were included in the non-lethals, and the non-lethals, through an error, contained in addition five too many chromosomes. The data of this table show that inversions occurred with approximately equal frequency in lethal and non-lethal chromosomes and in the two collections. The total for all groups combined is 8.0 per cent of inversions in 275 chromosomes.

*Inversions in Lethal and Non-lethal Chromosomes*

| Collection | Lethals tested | Inversions (%) | Non-lethals tested | Inversions (%) |
|---|---|---|---|---|
| 1938 | 56 | 4 (7.2) | 13 | 1 (7.7) |
| 1945 | 103 | 8 (7.8) | 103 | 9 (8.7) |
| Total | 159 | 12 (7.5) | 116 | 10 (8.6) |

Crossover tests showed that these 22 inversions were distributed 11 in the left arm of the chromosome and 11 in the right arm. In all cases there was consistently an almost complete lack of crossingover in the arm carrying the inversion. None of the other 253 chromosomes showed a consistent deviation from a normal amount of crossingover in either arm. The distribution of left and right arm inversions in lethal and non-lethal chromosomes was random.

Dr. Hinton examined 15 of the 1945 inversions cytologically. He found that the eight in the right arm were all the same inversion, which has breaks corresponding to those of the Nova Scotia inversion (Morgan, Bridges and Schultz, 1936). The seven in the left arm were also identical and seemed at first to fit the description of the Curly inversion. However, when it was found that Bridges had described another sequence, the *t* inversion, as differing from the Curly left arm inversion by only very minute cytological detail (actually by only one easily detectable band) an examination of the one remaining line of the left arm inversion in the present collection revealed that it is more probably the *t* inversion. Presumably the other six inversions were also the *t* inversion and not the Curly one, but this can never be known with certainty.

The 1938 inversions were not analysed cytologically. In view of the above considerations, however, it seems probable that they, too, represented the *t* and Nova Scotia inversions.

## Discussion and Conclusions

The two second chromosome inversions found in the local wild population of *D. melanogaster* apparently represent alternative gene sequences which at one or more points in the annual cycle of environmental conditions existing in the area have a selective advantage over the standard gene sequence. They are present in too large a proportion of the chromosomes to have accumulated as random mutations. A total of 1670 tests in this laboratory failed to produce a single inversion in either arm of the second chromosomes of flies herterozygous for 2-ple and the Canton wild chromosome. (These were reported as 835 tests in my 1945 paper, in which cognizance was not given to the fact that the 2-ple chromosomes were also being tested for inversions.) Nor are they likely to be the result of common parentage since the data on identical lethals indicate too low a frequency of sibs in the collection to account easily for so many identical inversions. Finally,

they do not appear to be deleterious in themselves, since the proportion of inversions in lethal chromosomes is the same as the proportion in non-lethal ones. Indirectly, then, the evidence is in favor of an occasional selective advantage of the inversion sequences. Direct evidence will be sought on this question in future collections from this area.

Both the *t* and Nova Scotia right arm inversions have a wide distribution in North American populations of *D. melanogaster*. Bridges found the *t* inversion in a wild stock from Texas, and in a mutant stock (with Florida background) from Swigert of this laboratory (Bridges and Brehme, 1944). It has been found at least two other times in laboratory mutant stocks of unknown sources. In addition, Dr. Hinton tentatively describes as *t* the left arm inversion of a double inversion chromosome extracted by the author from a Winter Park, Florida, 1942 collection. The other inversion in this chromosome he describes as the Nova Scotia right arm sequence. Bridges (Bridges and Brehme, 1944) found that the right arm inversions collected by Sturtevant in wild populations of Nova Scotia, Louisiana, Alabama and Cuba were all the Nova Scotia sequence. Warters (1944), making no reference to Bridges' descriptions, appears to have found both the *t* and the Nova Scotia right inversions in her abundant material from Mexico and the southern and western United States. She designates her right arm inversion as the equivalent of the one that Sturtevant found in his collections in the same area. While she considers that her left arm inversion is the Curly sequence, she was apparently unaware of the exceptionally close similarity of the *t* sequence. Bridges' data on the *t* inversion, especially his finding the *t* in a Texas wild stock, and his failure to find the Curly inversion in any material not derived from the original Curly stock make it seem much more probable that Warters' left inversion is the *t* sequence.

In all of her material Warters found only one left and one right arm second chromosome inversion. She reported extremely high frequencies of these inversions in some areas. In four instances, two for each inversion, the inversion chromosomes outnumbered the standard sequence in the chromosomes which she analysed. At least two other inversions have been reported in other American populations, a Nova Scotia left arm inversion (Sturtevant, 1931) and a Curly right arm one (Ward, 1923), both of which Bridges describes as easily differentiated from the others in salivary gland chromosomes. In a 1941 sample from Gallup, New Mexico, the author found a new right arm inversion which is apparently inseparable from a variable wing mutation, Notch-2. Hinton describes this as having breaks at approximately regions 50E and 54B on C. B. Bridges' salivary gland chromosome map (Bridges, 1935). It is not known how extensive this inversion may be in the Gallup population area. Warters had no collection from this immediate vicinity. This inversion, In(2R)G, is easily enough differentiated from the Nova Scotia right sequence so that she would have noted its presence in her material. Presumably, its range is restricted to the Gallup area.

Dubinin et al. (1937) report finding occasional Curly left and Curly right inversions in four of the 19 areas from which they collected Russian *D. melanogaster*, the two inversions appearing more often together than apart. It is doubtful if they were aware of the similarity of the Curly left and *t* inversions; but their figures of heterozygotes of the Curly stock chromosome and the Russian double inversion chromosome show that in both arms the inversions are identical. There can be no doubt that their wild population inversions are the true Curly sequences first found by Ward in a Michigan stock. Dubinin et al. report another right arm inversion, without an accompanying description or figure, which was found in low frequency in one of their 19

areas. The total frequency of second chromosome inversions in their material was always much lower than that reported by Warters, reaching a maximum of 4 per cent for one of the inversions in one area. Thus, there is a clear difference both in the kind of inversions and in the frequency with which they appear in the two continental areas, North America and Asia. Apparently the evolutionary trend in the second chromosome of *D. melanogaster* is different in the two continents.

It is of course obvious that further extremely careful and very laborious cytological work is needed to distinguish between the *t* and Curly left arm inversions in all areas studied. Also, additional areas should be studied in the northeastern United States and in Canada to see if the two Curly inversions are generally distributed in these areas or if they are, or were, to be found only in Ward's Michigan area. In addition, the distribution of the Nova Scotia left arm inversion needs to be determined in the northern part of the United States and in Canada.

No attempt will be made here to discuss in detail the distribution of inversions in the third chromosome in wild populations of *D. melanogaster*. By far the most common third chromosome inversions in Warters' material, A in the left arm and B in the right arm, appear to be identical with the Payne inversions described cytologically by Bridges. Their frequency of appearance in Warters' material is of the same high order of magnitude as the frequency of her two second chromosome inversions. Neither of them was found in any of the Russian collections. One of the third chromosome inversions found most frequently in the Russian material Warters believes to be identical with one she found in a few mountainous areas in the western United States. As Warters points out, there is a marked difference in the inversion content of the wild population third chromosomes in the two continents. The evolutionary trend is fully as different in the third chromosome as it is in the second.

From these considerations, it can be seen that the evolutionary trend in *Drosophila melanogaster* appears to be in significantly different directions in North America and in Russia.

## Summary

A collection of 190 *Drosophila melanogaster* second chromosomes extracted from the local fruit orchard area in 1945 contained 45.8 per cent of lethals and semi-lethals, similar to the percentage found in 1938 but significantly lower than that found in 1941. In crosstests the lethals proved to be 0.91 per cent identical, more than twice as high as in 1938, though not significantly different statistically. The apple crop was reduced by frost damage to the blossoms in both 1944 and 1945 to less than 5 per cent of normal and was produced only in widely separated orchard areas. This appears to have reduced the size of the breeding population of *D. melanogaster* by only a small amount at its point of minimum size, leading to the conclusion that at that point at least the flies are probably not dependent upon the fruit for existence.

The frequency of inversions was the same in 1938 and 1945, 8.0 per cent in 275 chromosomes. Non-lethal chromosomes contained as many inversions as were found in lethal chromosomes. No inversions were found in 1670 control tests. The wild inversions were distributed evenly between the two arms of the chromosome. Cytologically they were found by Dr. Taylor Hinton to consist of only two inversions, one for each arm, the Nova Scotia sequence in the right arm and probably the *t* (but possibly the Curly) in the left arm.

Examination of the distribution of inversions in wild populations of North American and Russian second and third *D. melanogaster* chromosomes shows marked differences in the two geographical regions which suggest that the evolu-

tionary trend within the species is different in the two continental areas.

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# A COMPARATIVE ANALYSIS OF THE CHROMOSOMES OF THE GUARANI GROUP OF DROSOPHILA

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## INTRODUCTION

In some families of plants and animals there is great uniformity of karyotype; in other families, sometimes even within a single genus, there is considerable variation. Where such variation exists, it is variation on an established theme, for karyotype like macromorphology arises as the result of various tendencies which preserve a somewhat unstable equilibrium within the organism. When it varies, it does not do so capriciously or arbitrarily. In groups where the usual taxonomic characters differ very slightly from species to species, chromosomal configuration may be a better guide to specific differences than the characters of external morphology.

Within the genus Drosophila there is considerable variation of karyotype from species to species. The haploid number of chromosomes varies from three to six, and individual chromosomes may be rods, dots, V's or J's in varying sizes and proportions. Certain general configurations are found widely spread throughout the genus; again, two species scarcely differing morphologically may have very distinct chromosomal complements. The several very similar species of the *virilis* group were first satisfactorily separated as a result of chromosomal analysis (Patterson, 1943).

The *guaraní* group consists of six species which on the basis of morphology are very closely related. They clearly belong to the subgenus Drosophila but are not closely related to any other known species of the genus. The present paper deals with the chromosomal configuration of these six species and the light which this chromosomal analysis sheds on the interspecific relationships within the group. Another paper dealing with the general interspecific relationships aside from the chromosomal configurations is in preparation and will be published shortly.

The six species of the *guaraní* group are the following: *guaraní* Dobzhansky and Pavan, *guarú* Dobzhansky and Pavan, *guaramunú* Dobzhansky and Pavan (Dobzhansky and Pavan, 1943), *griseolineata* Duda (Duda, 1925), *subbadia* Patterson (Patterson, 1943), and *guarajá*, species nova, described below.

### *Drosophila guarajá*, species nova

Male. Arista with 9 branches, sometimes with as many as 4 or 5 more very small ones. Second segment of antennae dull tan with darker area around bristles, third segment darker, more corneous in color. Front dull grayish tan, lightly pollinose, area between ocelli almost black, frontal lines velvety yellow-brown, converging and almost joining anteriorly. Anterior orbitals $\frac{3}{4}$ posterior, middle orbital minute. Two prominent oral bristles of approximately equal length. Face dull corneous tan, lightly pollinose. Carina very large, broad, flattened, not sulcate. Cheeks dull corneous tan, their width about $\frac{1}{3}$ greatest diameter of eye. Eyes deep maroon with a fine brown pile.

Achrostical hairs in six rows, very irregular. No prescutellars. Anterior scutellars divergent. Thorax dull dark tan, finely pollinose, without pattern. Pleurae much darker, almost walnut, less pollinose than dorsum of thorax. Anterior sternopleural $\frac{3}{4}$ posterior and much finer, middle about as long as anterior but still finer. Legs corneous tan shading to darker proximally. Prominent apical and preapical bristles on midtibiae; scarcely differentiated on fore and hind. One or two strong bristles at base of each tarsal joint. Some fine recurved hairs on fore tarsi.

Abdominal tergites shining black, deeper caudad of segment 4. On tergites 3 and 4 a corneous tan stripe along anterior margin about half width of tergite, far from reaching lateral margins, extended posteriorly on mid-dorsal line. On tergite 2 a narrower similar stripe without extension along mid-dorsal line. Abdominal sternites deep brownish gray. Membranous conjunctivae pale corneous.

Wings with slightly brownish tinge; cross veins very lightly clouded. Two prominent bristles of equal length at apex of first costal section. Third costal section with heavy bristles on its basal ½. Costal index 3.3–3.7; 4th vein index 1.5–2.1; 5th vein index 1.1–1.4.

Length of body 2.8–3.1 mm., wing 2.3–2.4.

Female. No recurved hairs on fore tarsi. Costal index 3.2–3.9; 4th vein index 1.5–1.8; 5th vein index 1.1–1.3. Length of body 3.1–3.5 mm., wing 2.4–2.5 mm.

Anterior Malpighian tubes two, simple, short common stalk; posterior two with ends apposed but lumen not continuous.

Testes pale amber yellow with 7 or 8 coils, sperm pump with two long diverticula. Ventral receptacle with about fifty kinks, diameter gradually decreasing distally. Spermatheca pear-shaped, purplish brown, heavily chitinized, especially in old flies, no indentation.

Eggs—four slender filaments: an anterior pair and a posterior pair arising about ⅓ of length of egg from anterior end, latter about length of egg, anterior pair slightly shorter. Length of egg about 0.5 mm.

Puparia—yellow-brown, often with an orange tint. Horn slender, almost invariably bent making measurement difficult, horn index between 5 and 6. Anterior spiracle with 18–20 very slender branches.

Chromosomes—metaphase plates show five pairs of chromosomes; one pair of large equal-armed V's, three pairs of rods of slightly different lengths, and one pair of small dots. The two pairs of longer rods often show satellites and appear to have subterminal centromeres. The sex chromosomes are evidently the smallest rods, as in plates of male larvae one chromosome of this pair shows a large satellite. The X never shows this satellite. The V's are necessarily autosomes. In this respect the species differs from all known others of the group, having five rather than six centromeres.

Salivary chromosomes show five long euchromatic strands and a dot. The chromocenter is very small.

Type—deposited at the American Museum of Natural History.

Distribution—the species has been collected only at the type locality, Campos de Jordão near the border of the states of São Paulo and Rio de Janeiro, Brazil.

Relationships—closely related to *D. guaramunú* and *D. griseolineata*, forming with these two species a natural sub-group within the *guaraní* group. The sub-group is characterized by the following among other characters: dark color of body and eyes, spheroidal rather than ellipsoidal abdomen in both sexes of well fed and well developed flies, and structure of Malpighian tubes—anterior with a short common stalk and posterior with ends apposed but without continuous lumen.

## Materials and Methods

Cultures of *guaraní, guarú, guaramunú* and *griseolineata* were obtained from the laboratory of Professor Dobzhansky who brought them from Brazil in 1943. A culture of *subbadia*, collected in Mexico, was obtained from Professor Patterson of the University of Texas. The culture of *guarajá* was obtained from the University of São Paulo in October, 1945. At the same time and from the same source an additional culture of *guaraní* and one of *guarú*, both from different localities than the original cultures, were also received.

All the species grow reasonably well in the standard Drosophila culture bottles, but the adult flies are very adversely affected by an advanced state of fermentation in the culture medium. To start a successful culture it is necessary to age flies for three or four days in one bottle and then transfer them to a fresh bottle; otherwise oviposition will probably not occur. Sometimes a second transfer in another three or four days is necessary. Flies left for more than three or four days in one bottle usually become stuck on the surface of the food medium. A group of flies which have begun to oviposit can be transferred from bottle to bottle every two or three days and will produce satisfactory numbers of eggs and larvae for from six weeks to two months. The addition of grated yeast to the bottles when the larvae are about half grown greatly increases the number of larvae which pupate. In culture bottles the flies breed best at relatively low temperatures, 17–20° C.

Aceto-orcein squash preparations were

made from brains of nearly full grown, feeding larvae. In these, metaphase and prophase figures of somatic mitoses were studied. The salivary chromosomes were studied in similar squash preparations of glands taken from larvae ready for pupation which had been raised at 17–18° C. in bottles provided with extra yeast.

## The Mitotic Chromosomes

### *guaramunú* sub-group

*Drosophila guaramunú.*—Metaphase plates (figures 1 and 2) show five pairs of rods and a pair of dots. Four pairs of rods are rather short but with some variation in length among them. All give evidence of subterminal centromeres and one pair often displays distinct satellites. The fifth pair of rods is more than twice as long as the others. These are sometimes alike and sometimes heteromorphic and are without much doubt the sex chromosomes. The X has two constrictions not far from the middle, setting off a central segment of about $\frac{1}{5}$ the total length. Sometimes the chromosome is bent at these constrictions, giving it the appearance of a V, but apparently the centromere is at one end or very near it and not at either median constriction. The Y, as observed in the plates, is a long rod often curved rather than bent. It contains one constriction about $\frac{1}{3}$ of its length from one end, and at the tip of the smaller segment there is a small but distinct satellite or bead. Prophases (figures 3 and 4) show the heterochromatin to be very largely concentrated in the two long rods, especially in the Y. The constrictions in the sex chromosomes visible at metaphase can be distinctly seen in most prophases.

*Drosophila griseolineata.*—In metaphase figures of this species (figures 5 and 6) there are also five pairs of rods and a pair of dots. The dots are distinctly smaller than those of *guaramunú*. Four pairs of rods are of approximately equal length and all are rather short. None bears a satellite and there is little evidence that the centromeres are anything but terminal. The fifth pair of rods is about twice as long as the others. Careful study shows these rods to be distinctly, if somewhat subtly, heteromorphic. One usually shows a submedian constriction and never a satellite. The other does not show any one definite constriction and does show a very definite satellite. The long rod with the satellite never occurs twice in the same cell; it is always paired with the one showing the constriction and having no satellite. The latter does often occur twice in the same cell. Therefore the long rod with the satellite is the Y, its homologue the X.

Prophase figures (figures 7 and 8) show the Y to be entirely heterochromatic and the X to contain some heterochromatic segments. The autosomes in general seem to contain very little heterochromatin. Thus the general chromosomal configuration of *guaramunú* and *griseolineata* is very similar although the total amount of heterochromatin seems to be smaller in the latter than in the former.

*Drosophila guarajá.*—The metaphase figures (figures 9 and 10) of this hitherto undescribed species differ from those of all other species of the group in showing five rather than six pairs of chromosomes. There are three pairs of rather short rods of slightly different lengths, a pair of V's and a pair of dots. The sex chromosomes must be the smallest pair of rods for while these are sometimes identical, they are also sometimes distinctly heteromorphic, one of them showing a very large, distinct satellite. This short rod with the large satellite never occurs twice in the same cell although its homologue often does. The short rod with the satellite must, therefore, be the Y and its partner the X. Both pairs of autosomal rods usually show very distinct satellites. Since the sex chromosomes have been identified as rods, the pair of V's must necessarily be autosomal. This offers an explanation of the reduced number of chromosomes in *guarajá*. Two autosomal rods have evidently become fused by

translocation with the resulting loss of one centromere.

Prophases (figures 11–12) bear out the deductions made from the metaphase figures. The Y appears to be the only chromosome which contains any appreciable amount of heterochromatin. It appears to be wholly heterochromatic and shows up with its large satellite clearly visible. The X appears to contain little heterochromatin which is reasonable because of its small size. The satellites of the two pairs of autosomal rods are also clearly in evidence. The dots appear in some figures to have condensed precociously, but whether or not they con-

Fig. 1. *guaramunú* female metaphase.—Fig. 2. *guaramunú* male metaphase.—Fig. 3. *guaramunú* male prophase.—Fig. 4. *guaramunú* female prophase.—Fig. 5. *griseolineqta* female metaphase.—Fig. 6. *griseolineata* male metaphase.—Fig. 7. *griseolineata* female prophase.—Fig. 8. *griseolineata* male prophase.—Fig. 9. *guarajá* male metaphase.—Fig. 10. *guarajá* female metaphase.—Fig. 11. *guarajá* female prophase.—Fig. 12. *guarajá* male prophase.

tain a large proportion of heterochromatin, is not at all clear. What appear to be dots in figure 11 are more probably the satellites of the adjacent pair of rods.

As was noted in pointing out the relationships of *D. guarajá,* the three species just discussed form a natural sub-group. They can be distinguished from each other morphologically, although the differences are rather slight, especially between *guaramunú* and *guarajá.* As to chromosomal configuration there are similarities between these species which set them off from the three species still to be discussed. In all of them the sex chromosomes are rods and in all the Y bears a distinct satellite. Passing now to *guarú, guaraní* and *subbadia* we come to three species which likewise form a distinct sub-group. They are set off morphologically from the species of the other sub-group, but among themselves they are extremely similar in morphology; and genically, as we shall see later, they are much more nearly alike than are *guaramunú, griseolineata* and *guarajá.*

### *guaraní* sub-group

*Drosophila guarú.*—Metaphase figures (figures 17 and 18) show six pairs of chromosomes: a pair of V's, four pairs of rods and a pair of dots. One pair of rods is perceptibly shorter than the others which are of approximately equal length. The rods all give evidence of having satellites or subterminal centromeres. The V's are of two kinds: one approximately equal-armed, one arm of which usually shows the equational split before the other, and one unequal-armed in a ratio of between 1 : 3 and 1 : 4 with often a secondary constriction in the long arm about one third of the way from the distal end. These heteromorphic V's are undoubtedly the sex chromosomes. Known male larvae always show one unequal-armed V and no cell has been seen which contained two unequal-armed V's. The equal-armed V is, therefore, the X and the unequal the Y.

Prophases (figures 19 and 20) confirm the subterminal location of the centromeres in the rods and show three pairs of rods with satellites. They also show the X chromosome with large heterochromatic sections, especially in one arm, and show the Y, the unequal-armed V, as entirely heterochromatic.

*Drosophila guaraní.*—This species is so similar in appearance to *guarú* that even one who has great familiarity with both species finds it extremely difficult to determine individual flies. Nevertheless, their chromosomes are unmistakably different. Metaphases of *guaraní* (figures 13 and 14) show six pairs of chromosomes: one pair of V's and five pairs of rods. The rods vary somewhat in length. Usually one pair is distinctly shorter than the others. All the rods appear to have satellites or slightly subterminal centromeres, and one pair seems to have the centromere so distinctly subterminal as sometimes to appear as a "J" with a short hook. The V's are about equal-armed, the arms being about as long as the longest rods. Often one arm shows a small satellite, and there is a secondary constriction in each arm a short distance from the centromere. There is nothing in the metaphase plates to indicate which pair of chromosomes constitutes the X and Y.

Prophase configurations (figures 15 and 16) bring to light two very interesting facts. First, the pair of V's appears to be composed largely of heterochromatin. Sometimes one V is apparently heterochromatic throughout while the other appears to be so in one arm only. This makes it clear that the V's are the sex chromosomes, that the Y is entirely heterochromatic and that the X is euchromatic in one arm only. Second, one of the shorter pairs of rods—whether or not the shortest is not clear—appears to be wholly heterochromatic with a dot-like satellite at one end. This observation is of particular significance as it suggests an explanation for the absence of a pair of dot-like chromosomes which are pres-

ent in *guarú* and are so common among species of Drosophila. If a segment of heterochromatin had been translocated to the pair of dot-like chromosomes of *guarú*, we should have a configuration of two V's and five pairs of rods, precisely what we have in the metaphase plates of *guaraní*.

*Drosophila subbadia*.—This species closely resembles *guaraní*, but is more easily distinguished from it than is *guarú*. Its metaphase figures (figures 21 and 22) are very similar to those of *guaraní*. There are five pairs of rods and sometimes a pair of equal-armed V's. Other cells show one equal-armed V paired with

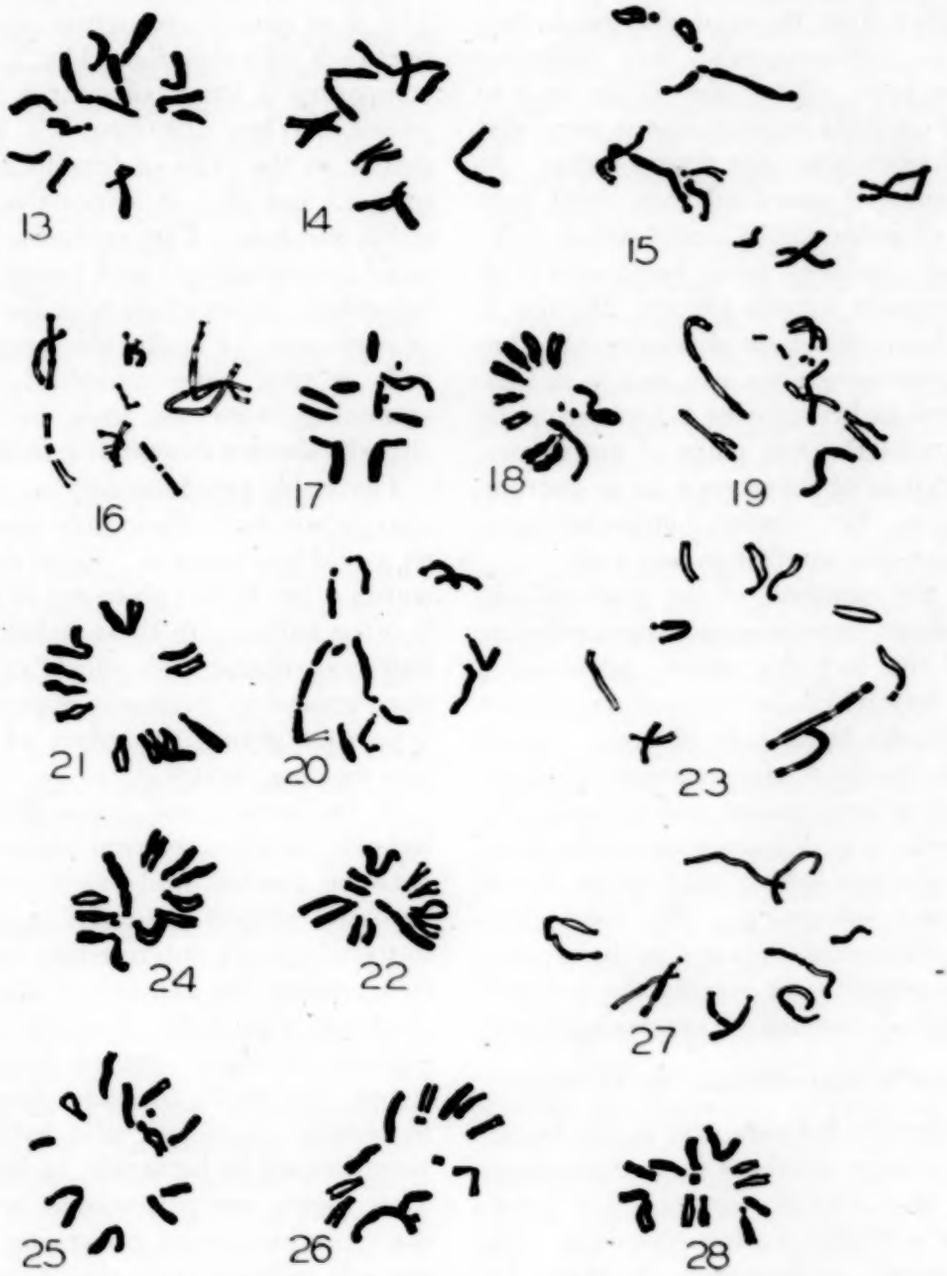


Fig. 13. *guaraní* metaphase.—Fig. 14. *guaraní* metaphase.—Fig. 15. *guaraní* prophase.—Fig. 16. *guaraní* prophase.—Fig. 17. *guarú* female metaphase.—Fig. 18. *guarú* male metaphase.—Fig. 19. *guarú* male prophase.—Fig. 20. *guarú* female prophase.—Fig. 21. *subbadia* female metaphase.—Fig. 22. *subbadia* male metaphase.—Fig. 23. *subbadia* prophase.—Fig. 24. *guarú* X *subbadia* male metaphase.—Fig. 25. *guarú* X *subbadia* female metaphase.—Fig. 26. *guarú* X *subbadia* female metaphase.—Fig. 27. *subbadia* X *guarú* female prophase.—Fig. 28. (*guarú* X *subbadia*) X *guaraní* male metaphase.

a very long rod with two constrictions, one at about the middle and the other about half way between this and one end. The long rod is evidently the Y chromosome as it is found in cells of larvae known to be male. Sometimes it is bent at the constriction nearest one end making it an unequal-armed V similar to the Y of *guarú*. The chromosomes of *subbadia* differ from those of *guaraní* in that the sex chromosomes are definitely heteromorphic. They also differ in that one pair of rods in *subbadia* is very distinctly longer than the four others. As in *guaraní* and *guarú* all rods show indications of subterminal centromeres. No detatched satellites have been observed.

In prophase figures (figure 23) the X and Y have not been distinguished, but the sex chromosomes can easily be distinguished as being more heterochromatic than four of the five pairs of autosomes. The fifth pair of autosomes, as in *guaraní*, appears to be wholly heterochromatic with a dot-like satellite at one end.

Thus the members of the *guaraní* subgroup show chromosomal similarities in spite of the fact that *guarú* possesses a pair of dots which is replaced in *guaraní* and *subbadia* by a pair of rods. In all three species the X chromosomes are large V's, and in two, *guarú* and *guaraní*, the Y is also a large V. In general the autosomal rods are longer than those in the *guaramunú* sub-group. The total chromosomal material appears to be greater and this seems to be largely the result of an increased amount of heterochromatin.

### Mitotic Chromosomes of Hybrids

Interspecific hybrids may easily be obtained in large numbers by mating *guarú* females and *subbadia* males. The reciprocal cross produces a few offspring. The chromosomal configuration in these hybrids is of great interest for here we are able to see chromosomes of the two species side by side in the same cell. The most striking characteristic of the metaphases (figures 24, 25 and 26) is that they show an uneven number of rods. Just as would be expected from the separate analysis of the chromosomes of the two species, one dot has as its homologue a rod. In some cases the dot element of this rod appears slightly separated from the heterochromatic portion of the chromosome; in other cases the dual make-up of this chromosome is merely suggested. In plates from known male larvae (figure 24) it is easy to recognize the long tripartite Y of *subbadia*. The V which accompanies it must, of course, be that of *guarú*. When the two X's appear together in the cells of female larvae (figures 25 and 26), it is possible to distinguish the two. The *subbadia* X is more nearly equal-armed and tends to have a secondary constriction near the distal end of each arm. Finally, the longest pair of rods is also heteromorphic: the longer stemming from the male *subbadia*, the slightly shorter from the *guarú* female.

Favorable prophase figures (figure 27) also demonstrate these differences exactly as would be expected. Since dot chromosomes often fail to show up in prophases, it often happens in these hybrids that the heterochromatic rod which contains the dot element in *subbadia* appears without a partner giving the effect of an eleven chromosome figure.

It has been found impossible to obtain hybrids between *guaraní* and *subbadia* or between *guaraní* and *guarú*, even though in many respects *guaraní* appears to be morphologically intermediate between the two. Since for reasons of chromosomal study it was very desirable to involve *guaraní* in some sort of liaison, an attempt was made to mate *guaraní* males to female *guarú-subbadia* hybrids which were known to be fertile in back-crosses. This highly unconventional arrangement does produce some off-spring, and both somatic mitoses and salivary gland chromosomes of such triple hybrid larvae have been studied.

Metaphases of these hybrids (figure 28), which are almost invariably males, show necessarily a *guaraní* Y. In all plates sufficiently favorable to make de-

termination possible, the X appears to be that of *guarú*. All the plates which have been examined show one dot, which must have come from *guarú*, paired with a rod which must be attributed to *guaraní*. In some cases one of the autosomal rods appears perceptibly longer than its homologue and is probably the very long autosomal rod seen in metaphases of *subbadia*. The fact that no females are produced in this cross suggests that there is an incompatibility between the *guaraní* X and the X of either *guarú* or *subbadia*. Furthermore, the absence from male larvae of the *subbadia* X and of that *subbadia* autosome which is largely heterochromatic strongly suggests that only a restricted number of the possible chromosomal combinations is viable and that the others fail to develop.

### Salivary Chromosomes

Salivary gland cell preparations of all species of the group are unfavorable for detailed observation. Often the nuclei of the cells do not break when the squash preparations are made, and when they do, the salivary chromosomes generally remain snarled around each other and many of the strands are snarled back upon themselves making it very difficult to find the free ends. If enough trials are made, however, some favorable cells can be found.

The salivary chromosomes of the species of the *guaramuná* subgroup have been investigated only for general configuration and all three species show five long strands and a dot. This is what one should expect from the metaphases of *guaramuná* and *griseolineata*, one long strand for each paid of rods and a euchromatic dot corresponding to the pair of dots. The case of *guarajá* is particularly interesting because it shows that both arms of the V's must be euchromatic and also because its chromocenter is strikingly small. This bears out the evidence of the prophases that there is very little heterochromatin in the entire chromosomal complement of the species. Since what little heterochromatin there is seems to be in the Y, one would expect the chromocenter in the cells of male larvae to be distinctly larger than those in the cells of female larvae; but this has not been definitely established.

In general configuration the salivary chromosomes of the *guaraní* sub-group also show five long strands and a euchromatic dot. When the chromocenter is broken, four strands pull free with a small amount of heterochromatin adhering to their bases and the rest of the chromocenter remains attached to one strand which is associated with the nucleolus. This latter strand undoubtedly represents the X for it is paler in the cells of male larvae. The euchromatic dot is usually embedded in or attached to the large mass of heterochromatin adhering to the X. These observations on the salivary chromosomes of this subgroup confirm the deductions that the metaphase V's are the sex chromosomes, that the X is euchromatic in only one arm, and that in *guaraní* and *subbadia* the fifth pair of rods is largely heterochromatic and contains only a dot-like euchromatic element.

Of all the pure species of the group, direct and intensive study of the salivary chromosomes has been carried out only in the case of *guarú*. Here it has been possible to identify the tips and bases of the five long strands, although the identification of more than two strands in any one cell is possible only rarely.

The most interesting thing about the *guarú* salivaries is the number of inversions which they show. It has not been possible to make an exhaustive list of these because the chromosomes are so infrequently disentangled. However, certain of them have been definitely identified. The diploid X appears to contain several inversions, but this chromosome is almost invariably snarled and contorted to such an extent that only one small inversion near the base has been definitely isolated. The four long autosomes afford somewhat more favorable observations. They have been lettered A, B,

C and D as identified by their tips and bases. Chromosome A (figure 29) is usually spread out more favorably than any other. It has a very distinctive tip and a subterminal bulb. It often contains the large double inversion shown in the figure. It lies in the basal half and has one large and one small loop. This inversion has been seen many times. Chromosome B (figure 32) is much less frequently favorable for observation, but the complicated double inversion shown near the tip and the large single inversion to be seen near the base have been identified. Chromosome C is also usually unfavorable for observation, but apparently it contains a subterminal single inversion. No inversions have been definitely identified in chromosome D, and no evidence of rearrangement has been observed in

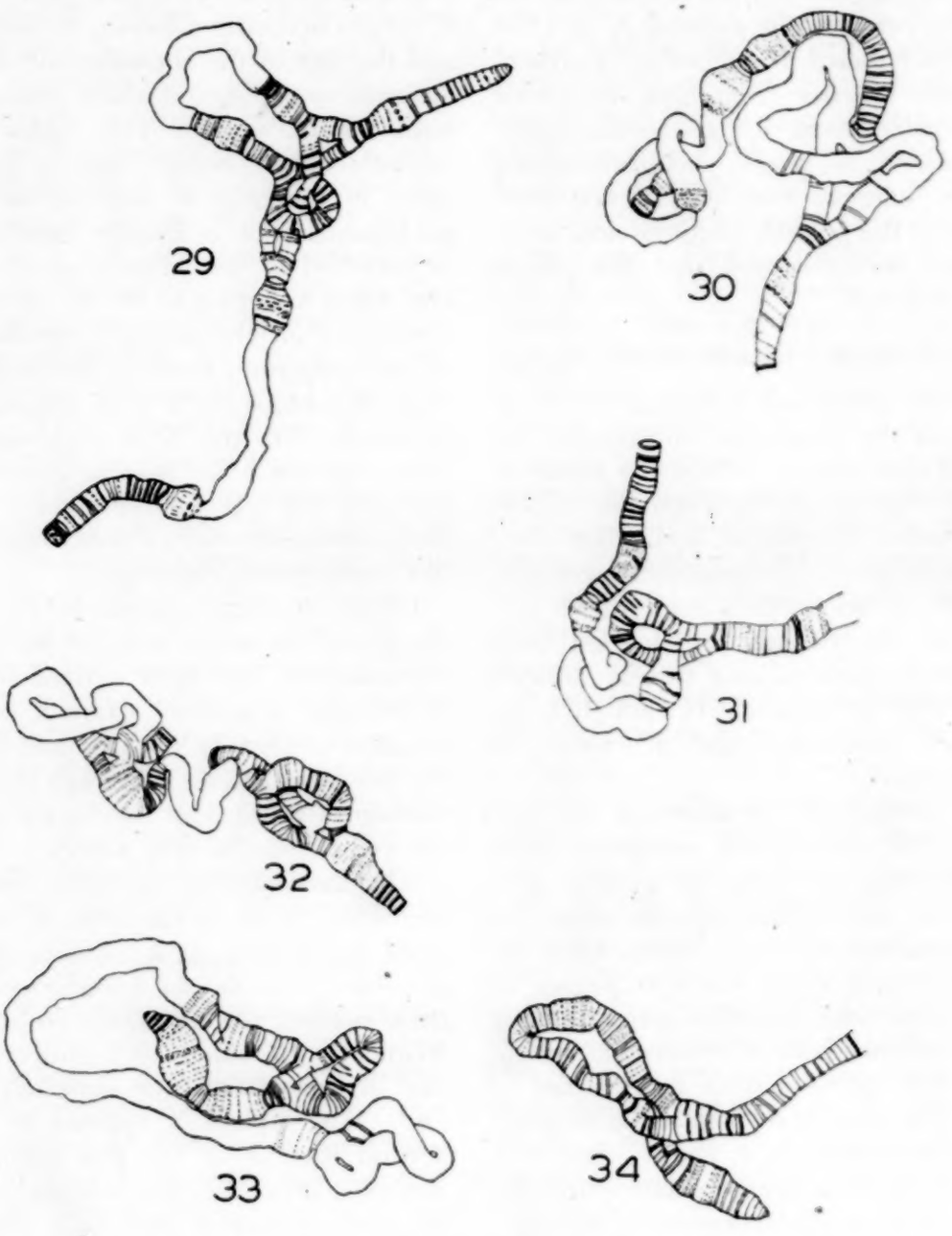


Fig. 29. *guarú* salivary chromosome A.—Fig. 30. *guarú* × *subbadia* salivary chromosome A.—Fig. 31. *guarú* X *subbadia* salivary chromosome A.—Fig. 32. *guarú* salivary chromosome B.—Fig. 33. *guarú* X *subbadia* salivary chromosome B.—Fig. 34. *guarú* X *subbadia* salivary chromosome B.

the dot, chromosome E. There are undoubtedly other rearrangements. Some of them have been observed, but under circumstances when it was impossible to tell in which chromosome or in what part of it they lay or when they were not sufficiently clear to make identification possible. The number and persistence of these inversions are the more remarkable since they have all been found in the strain of *guarú* descended from a single female collected at Amalia.

A second strain of *guarú* collected at Bertioga has not been intensively studied for inversions. But examination of the glands of four larvae showed inversions in chromosomes A, B and C. Not one of these appeared to be identical with any inversion known in the Amalia strain. One of them, a small subterminal inversion in chromosome A including the bulb, was particularly clear and unmistakable.

Another interesting phenomenon observed in the salivary chromosomes of *guarú* concerns the boundary between the euchromatin and the heterochromatin at the base of the X. This is not the same in all cells. A rather sizeable segment which shows discrete discs in some cells is entirely assimilated to the chromocenter in others, and various intermediate conditions can also be found. This is precisely the condition described by Dobzhansky (1944) in the salivary chromosomes of *D. pallidipennis,* in this case also in the X.

### Salivary Chromosomes of the Hybrids

The salivary chromosomes of the *guarú-subbadia* hybrids are even more interesting than the mitotic ones. Perhaps the most striking fact is that these chromosomes show excellent pairing, quite as intimate as in either pure species, and there is no evidence that any part of any chromosome fails to find its effective homologue. As must follow from these observations, the general configuration in these salivaries is precisely the same as in either pure species.

Since numerous inversions occur in the salivaries of the pure species *guarú,* they must also appear in the hybrids. Actually many inversions are found there. Some of them cannot be identified with any known inversions occurring in *guarú.* This is not at all surprising since the chromosomes of each of two species might be expected to contain gene arrangements not shared by both. Some inversions in the hybrids, however, can be identified with some which exist in the pure species of *guarú,* and these are of very great interest. The large double inversion which is often seen in the basal half of chromosome A of *guarú* has never been found as such in the hybrids. Nevertheless, the two elements of this inversion can be found separately in the hybrids as single inversions (figures 30 and 31). These facts can have but one meaning. The laboratory strain of *guarú* (Amalia) contains two gene arrangements of chromosome A, neither of which could have come into being from the other without the existence of an intermediate arrangement which apparently is not present. This missing arrangement is, however, present in the laboratory strain of *subbadia.* An exactly analogous situation occurs in the subterminal segment of chromosome B. The double inversion found there in pure *guarú* turns up in the *guarú-subbadia* hybrids as two separate single inversions (figures 33 and 34).

The intimacy of pairing and the facts concerning gene arrangements in the *guarú-subbadia* hybrids establish incontrovertibly that the two species have at some time been parts of one interbreeding population.

The salivaries of the triple hybrids, unlike those of the pure species or of the *guarú-subbadia* hybrids, show a substantial amount of failure of pairing. However the pairing is surprisingly complete, more so than is commonly the case in hybrids between two species of Drosophila. Providentially, one cell was found in which all the chromosomes were well spread out and could be identified. It

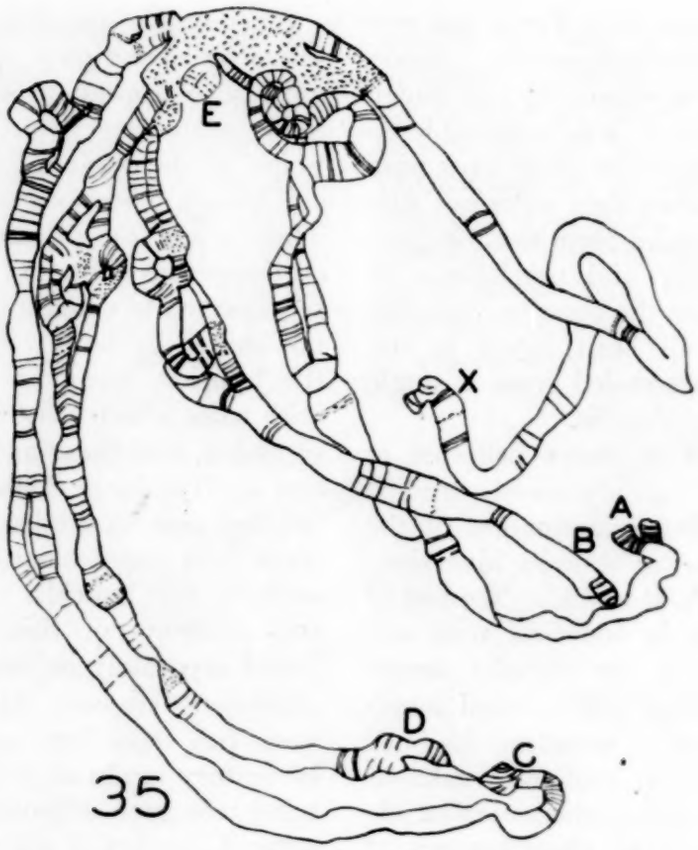


FIG. 35. (*guarú* X *subbadia*) X *guaraní*. Drawing of salivary gland cell.

was drawn and photographed (figures 35 and 36) and shows surprisingly few rearrangements. In chromosome A there appear to be three inversions in the basal portion, the same region where cells of *guarú* often show a double inversion. In chromosome B there seems to be a double inversion in the basal region near the area where a single inversion has been found in *guarú*. Chromosome C shows a subbasal single inversion, very possibly the same as has been found in *guarú-subbadia* hybrids. Chromosome D shows a double inversion in the basal portion. This is the only case in which a rearrangement has been seen definitely in this chromosome.

In the triple hybrids the tip of chromosome A shows a very interesting formation. This is the tip which is most easily identified in both *guarú* (figure 37) and *subbadia* and it appears unchanged in hybrids between them. In the triple hybrids (figure 38) this tip is very often unpaired for a short distance. Sometimes, however, as in the figure, it is paired at the extreme tip and then unpaired for a very short distance immediately behind. Careful examination of this unpaired region brings to light some minute differences in the bands there. These differences may be the result of a very short inversion, a very small duplication, or some other alteration of a few bands in this restricted area. At any rate while the tip of this chromosome is identical in *guarú* and *subbadia*, the *guaraní* tip differs in some very subtle way. The differences observed here are very similar to those discussed by Metz (1937 and 1938) in the salivary chromosomes of Sciara where they seem to be very common. As Metz notes, indications of similar structures have been found in certain other cases of Drosophila hybrids, although they seem to be much less common there than in Sciara.

Thus the chromosomal analysis of

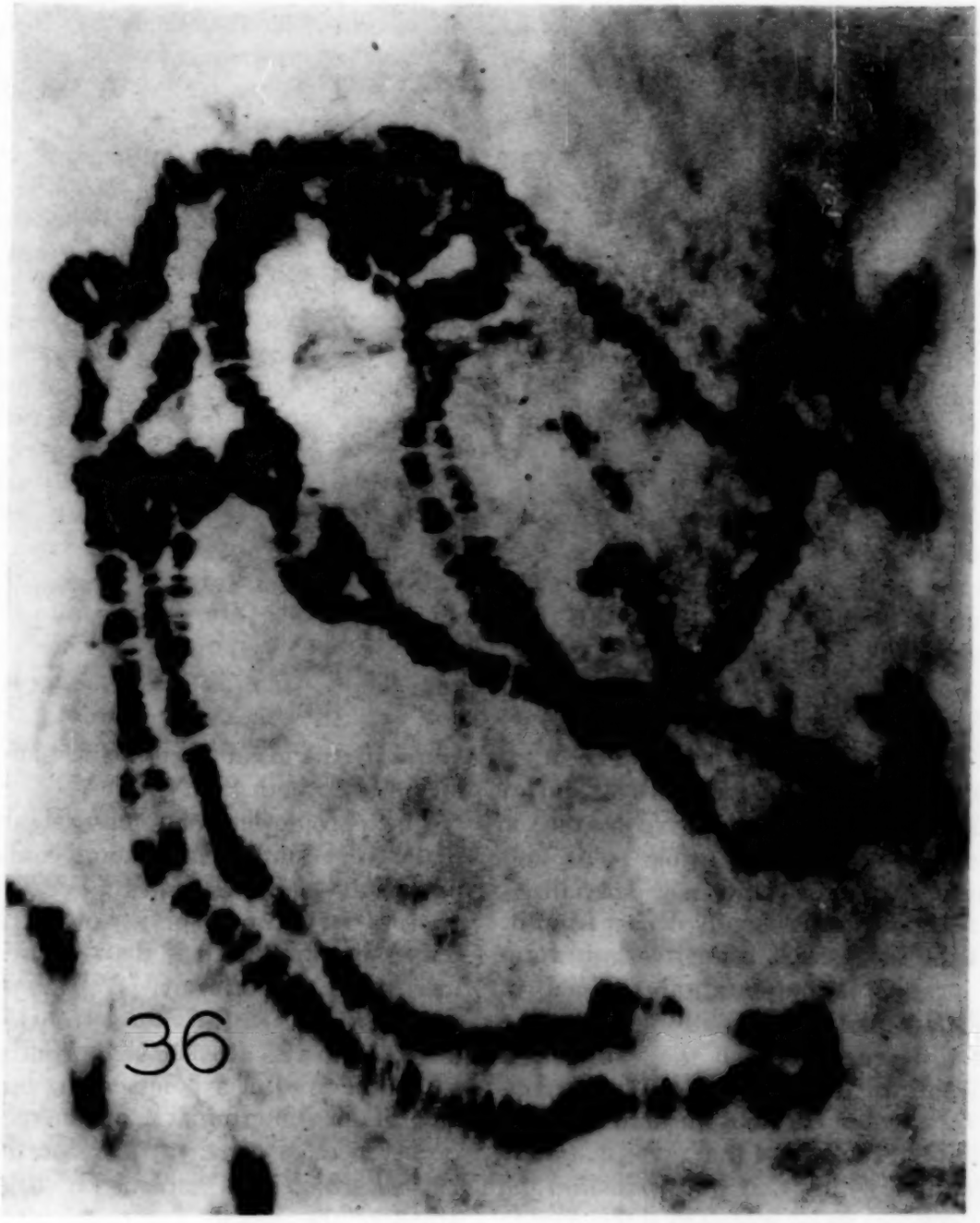


FIG. 36. Photograph of same cell figured in figure 35.

these three species demonstrates that *guarú* and *subbadia* which differ most in general appearance are actually more nearly identical genically than either is with *guaraní*, although in general aspect the latter appears to be intermediate between the two. Nevertheless, the three species are so similar genically that an individual containing a chromosome complement drawn from all three species can carry its development successfully to the adult state. One must conclude that not only *guarú* and *subbadia* but all three species have at some time constituted one single interbreeding population.

## DISCUSSION

The chromosomal configuration at metaphase which is most widely distributed throughout the genus Drosophila consists of five pairs of rods and a pair of dots. This configuration is commonly

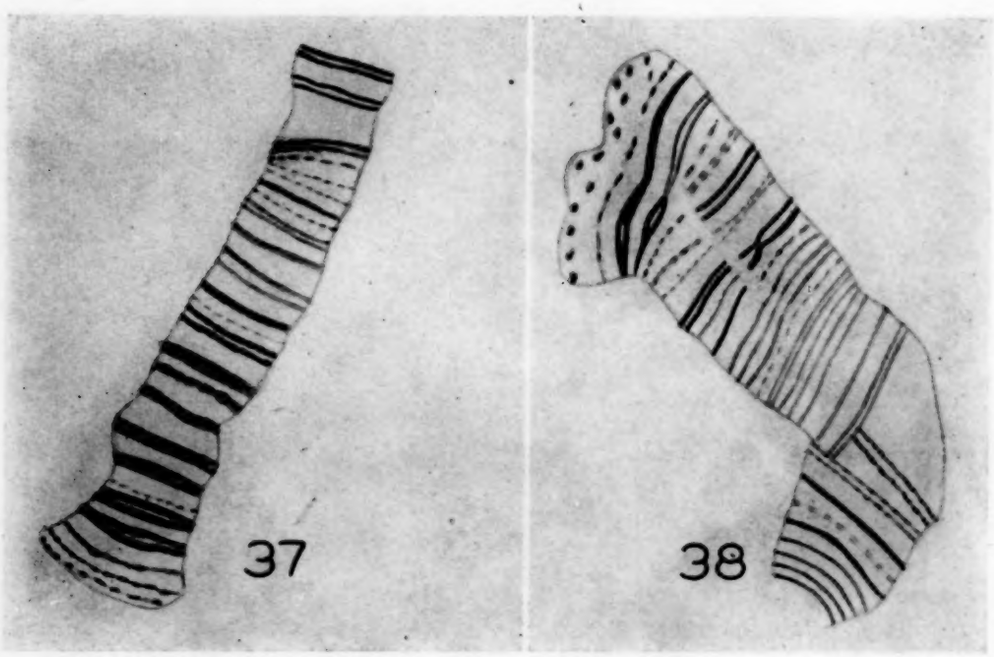


FIG. 37. *guarú*. Tip of salivary chromosome A.
FIG. 38. (*guarú* X *subbadia*) X *guaraní*. Tip of salivary chromosome A.

believed to be a generalized one because, among other reasons, all other known configurations can be derived from it through a series of plausible steps and because the most obviously specialized and aberrant forms usually depart from it.

Of the six species in the *guaraní* group this primitive configuration is seen in both *griseolineata* and *guaramunú*. The chief difference between the two lies in the length of the pair of rods which constitutes the sex chromosomes. In *guaramunú* both the X and the Y are distinctly longer than they are in *griseolineata*. This appears to be merely a difference in the amount of heterochromatin. The Y in both species seems to be entirely heterochromatic; the X of *guaramunú* shows a substantial heterochromatic segment while that of *griseolineata* gives evidence of very little. It is easy to imagine the origin of the *guaramunú* configuration from that of *griseolineata*. The Y could be lengthened by one or more duplications; the X by one or more translocations to it of heterochromatic sections from the Y. Or the *griseolineata* configuration could arise from that of *guaramunú* by the loss of heterochromatic segments from both the X and Y.

The chromosomes of *guarajá* are actually very similar in general configuration to those of *griseolineata* even though *guarajá* is the one species of the group possessing five rather than six centromeres. The autosomal V of *guarajá* must have arisen through a translocation of one rod which had lost its centromere to another rod at a point on the opposite side of its subterminal centromere. The occurrence of such a translocation in *griseolineata*, if accompanied by a lengthening of the X and Y through the addition of heterochromatic segments, would give a configuration almost identical with that found in *guarajá*.

It is interesting to note that the three species *guaramunú*, *griseolineata* and *guarajá*, which are very similar in so many respects, all have definite satellites on the Y chromosome.

Of the three remaining species, *guarú* is less different than the other two from the three just discussed. The chief difference lies in the fact that here the sex chromosomes are V's. A long rod-

shaped Y could become a V by the simple expedient of a pericentric inversion. Such has evidently occurred in *guarú* giving a V with unequal arms. The X could become a V either by a pericentric inversion or by the translocation of a heterochromatic segment from the Y to the short side of a subterminal centromere.

Passing to *subbadia,* the chief difference between this species and *guarú* is the replacement of the dot chromosomes by another pair of rods. We know that this was achieved by the translocation of a heterochromatic segment to the dots. Again, the most likely explanation is a translocation of an acentric fragment from the Y. An interesting point is provided by the pair of autosomal rods of *subbadia* which are so definitely longer than the corresponding pair in *guarú* and in *guaraní.* It may be that these rods have been lengthened in *subbadia* by another translocation of heterochromatin. The prophase (figure 27) of the *guarú-subbadia* hybrid suggests this explanation by the heteromorphism of the pair of chromosomes at six o'clock. But the pair at seven o'clock is also distinctly heteromorphic, and the prophase of *subbadia* (figure 23) does not clearly show any pair of autosomes, except those replacing the dots, as definitely more heterochromatic than the others.

The chromosomes of *guaraní* differ from those of *subbadia* largely in the greater total length of the Y which has become an equal-armed V. This change could easily have come about either by duplication or by translocation.

Within the six species of the group all major differences of chromosomal configuration can, with one exception, be explained by differences in amount and arrangement of heterochromatin. This is true for example of two species of which the metaphase plates present such completely different pictures as do those of *griseolineata* and *guaraní.* In fact the six species can be arranged according to the increasing relative amount of heterochromatin in the following order: *guarajá, griseolineata, guaramunú, guarú, subbadia* and *guaraní.* The one exception is the presence of the autosomal V in *guarajá;* and this is explainable as a comparatively simple translocation of euchromatic rather than heterochromatic material.

From the above analysis it seems safe to make the generalization that differences of chromosomal configuration between two species indicate little as to differences of genic balance. Differences of general configuration are evidence of degree of relationship only to the extent that one can estimate the probability of the occurrence of the chromosomal breaks which must be postulated to account for the differences. Two species having very different chromosomal configurations may nevertheless have very nearly identical genic make-up, *viz. guarú* and *subbadia.* The converse, that two species with apparently identical chromosomal configurations may be worlds apart in genic balance, is, of course, a truism.

## Acknowledgments

The author wishes to acknowledge his deep indebtedness to Professor Th. Dobzhansky who suggested the present study, initiated the author into the intricacies of Drosophila techniques, provided the cultures used, made his laboratory available and gave generously time, suggestions and inspiration. Thanks are also due to Professor C. Pavan of the University of São Paulo for valuable aid and suggestions during the course of the work.

## Summary

The chromosomes of the six species of the *guaraní* group of Drosophila are described and compared in metaphase and prophase of somatic mitoses and in salivary cells. Noticeable differences in metaphases and prophases can, with one exception, be explained as differences in amount and arrangement of heterochromatin. In one case the difference is the result of the loss of a centromere and the

fusion of two chromosomes. The salivary chromosomes bear out these conclusions.

Three of the species can be involved in crosses producing hybrids which make possible a more exact comparison of the chromosomes of these three species in metaphases, prophases and salivaries.

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# ECOLOGY AND THE EVOLUTION OF SOME HAWAIIAN BIRDS

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The land animals and plants of isolated archipelagos have evolved from a few straggling colonists which crossed wide expanses of ocean by occasional accidents of dispersal. Many groups numerous on continents are entirely absent thus leaving ecological niches vacant and setting the stage for rapid adaptive radiation. One example of adaptive radiation in birds is the Geospizidae or Galapagos finches, which Darwin studied. Even more remarkable in this respect are the Hawaiian Honeycreepers (Drepaniidae). During fifteen months in the Hawaiian Islands I studied these birds in the field and in the collection of the Bishop Museum, Honolulu. Some of the conclusions reached below are foreshadowed in the work of Dr. R. C. L. Perkins (1903, 1913), the greatest authority on the Hawaiian fauna. The present paper analyzes some examples of adaptive modification among Hawaiian birds and suggests that competition between related sympatric species with similar ecological requirements has been a major cause of such modifications.

## *Phaeornis*

The thrush family (Turdidae) is represented in Hawaii by one endemic genus, *Phaeornis,* containing two species. One species, *P. obscurus,* was represented by a different subspecies on each of the islands except Maui, where the genus is absent (some of the subspecies are now extinct). The other species, *P. palmeri,* occurs only on Kauai, the most isolated island of the group (figure 1). Presumably the first *Phaeornis* stock to reach Kauai was so isolated that differentiation to the specific level resulted, giving rise to *palmeri.* Later the widespread *P. obscurus* again colonized Kauai successfully. Such speciation by double invasion on suitably located islands is common in birds (Mayr, 1942:173). On the other Hawaiian islands, which are less isolated, we may assume that stragglers of the thrush have wandered from island to island frequently enough to keep differentiation below the specific level.

*Phaeornis obscurus* feeds on berries and fruits but sometimes takes insects. *Phaeornis palmeri* is chiefly insectivorous. Perkins (1903:377) found in the stomachs of specimens of this species ". . . the large hard weevils of the genus *Rhyncogonus,* and these unquestionably form a large part of their food. Spiders and caterpillars are also eaten, and the insectivorous habits of the species are strongly contrasted with the berry-eating propensity of the other members of the genus [i.e., the races of *obscurus*]. . . . Owing however to their totally different habits there is little or no competition between the two forms, as at present constituted." Since the five races of *obscurus* do not differ, so far as known, in habits, it is probable that the different habits of *palmeri* were accentuated by competition with a congeneric species after the second colonization of Kauai by *Phaeornis.*

Lack (1944) in a summary of information on ecology and speciation in birds points out that when two species with similar requirements meet in the same area those individuals which differ most

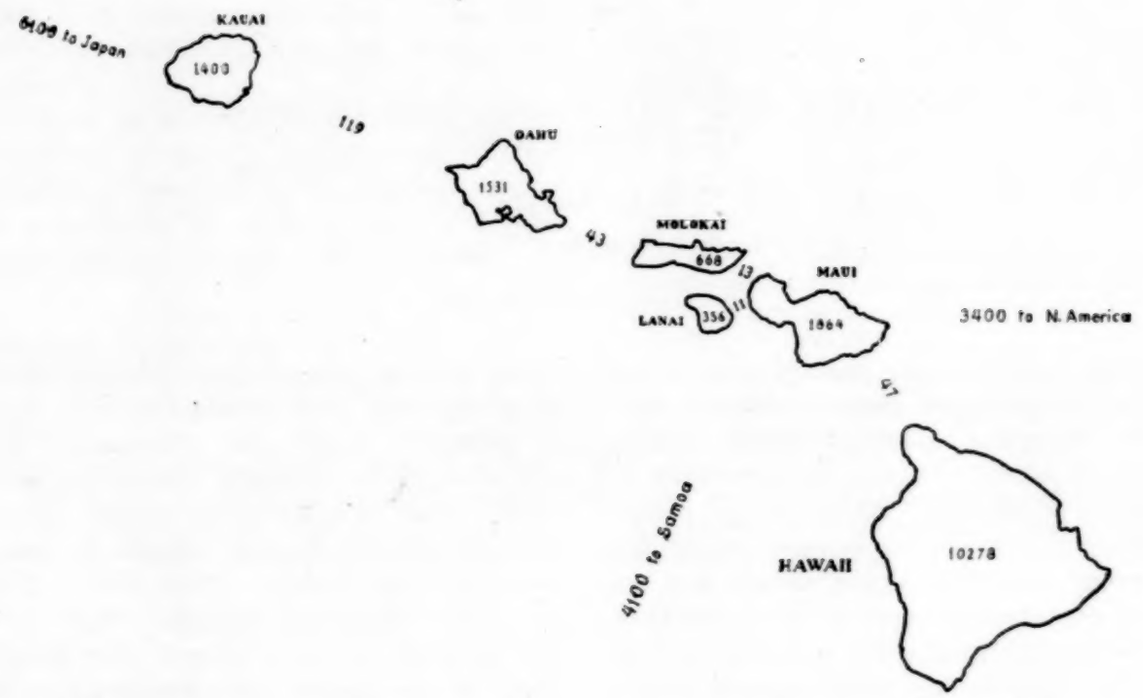


FIG. 1. Map of Hawaiian Islands. Distances between islands in kilometers; areas of islands in square kilometers.

in feeding habits (or in structures concerned with feeding such as the bill) will have a selective advantage. This differential selection will tend to draw the two species apart, especially where suitable empty ecological niches exist. Some slight difference in requirements must exist at the time two such species first overlap since ecologists consider it virtually impossible that two species with identical requirements (if such ever evolve) could exist together for any length of time.

In *Phaeornis* the morphological differences between the two forms occurring on Kauai are not known to be adaptive. One is considerably smaller than the other, a common difference between pairs of very similar (sibling) species which is probably adaptive (Lack).

## ADAPTIVE EVOLUTION IN THE DREPANIIDAE

The following examples are from the family Drepaniidae. The bills of most of the species mentioned are shown in figure 2.

### *Loxops*

The genus *Loxops* contains two closely related species, *L. virens* and *L. parva*. They are often placed in a separate genus *Chlorodrepanis*. As in the thrushes, one of these species (*virens*) is found throughout the islands while the other, which was apparently derived from it, is restricted to Kauai. *L. virens* searches for insects among foliage; it also uses its short decurved bill to extract nectar and small insects from flowers; occasionally it digs away bits of loose bark with its bill. The Kauai subspecies (*L. virens stejnegeri*) has a noticeably heavier bill and digs more in bark and moss while seeking insects. Munro (1944:100) wrote of it: "I noted particularly in skinning that the muscles on the back of its head were more strongly developed than in the other Kauai birds and its skull more heavily built. This development was evidently occasioned by its habit of digging in the bark of trees to a greater extent than any of the other Kauai birds; or other *amakihis* [i.e., other races of

*virens*]." *L. parva,* on the other hand, is a tiny bird with a small, almost straight bill. Munro (1944:104) wrote of it: "It usually gathers its insect food among the twigs and leaves but sometimes in the loose bark. It is very fond of visiting the koa [*Acacia koa*] flowers."

In *Loxops* the two Kauai forms have

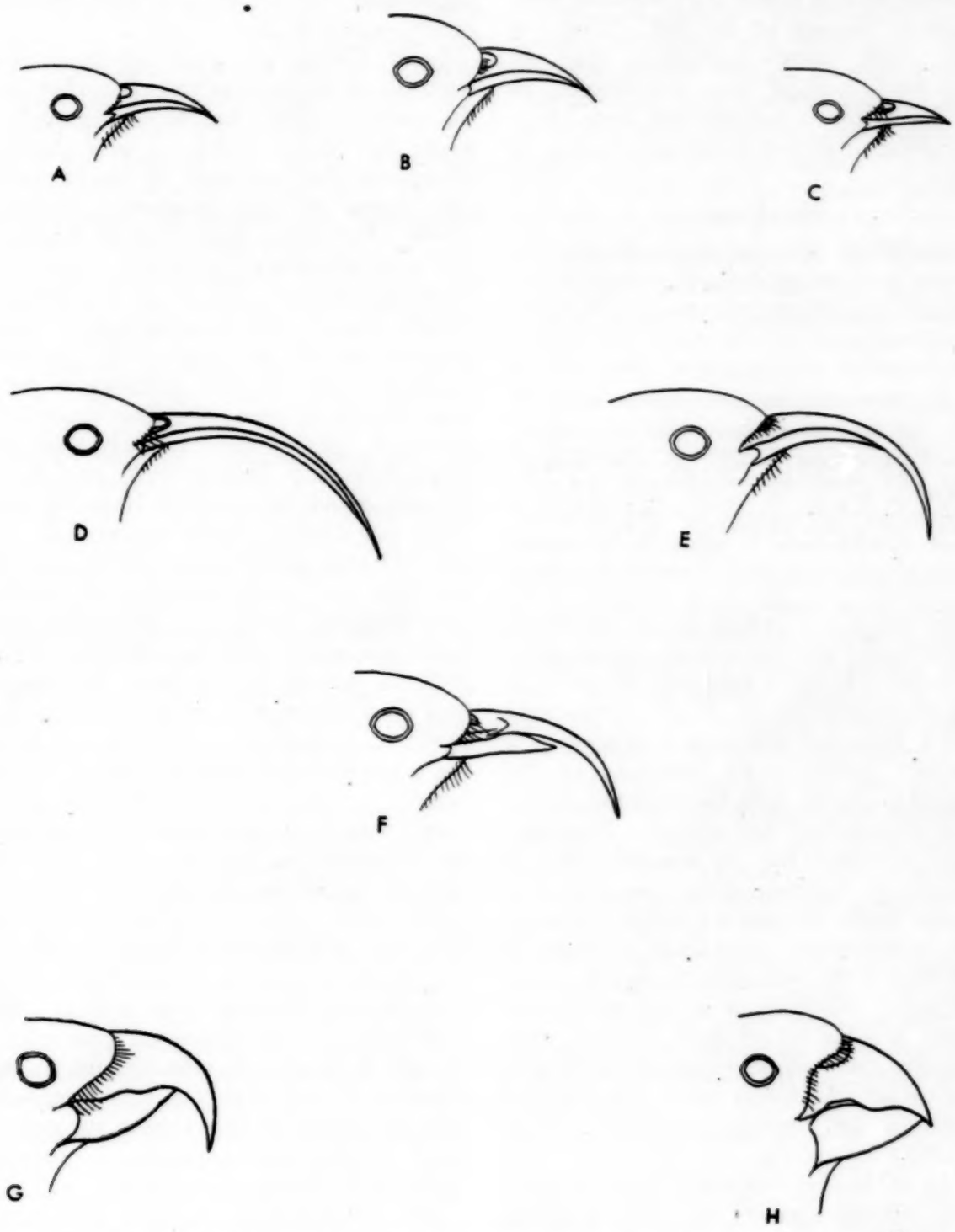


FIG. 2. Bills of (A) *Loxops virens chloris;* (B) *Loxops virens stejnegeri;* (C) *Loxops parva;* (D) *Hemignathus o. obscurus;* (E) *Hemignathus lucidus hanapepe;* (F) *Hemignathus (lucidus) wilsoni;* (G) *Pseudonestor xanthophrys;* (H) *Psittirostra kona.* From drawings by F. W. Frowhawk in "Avifauna of Laysan and the Hawaiian Possessions" by Walter Rothschild (London, 1893–1900).

both changed somewhat, as compared with the more generalized races of the ancestral species occurring on the other islands. In *stejnegeri,* at least, the changed feeding habits are correlated with adaptive changes in the bill. Thus we may, with more confidence than in *Phaeornis,* suggest that modification of the Kauai forms has resulted from interspecific competition following overlap in range.

### *Hemignathus*

The genus *Hemignathus* contains two species groups (superspecies). Their geographical distribution is shown in table 1.

TABLE 1

| | *Hemignathus obscurus* | *Hemignathus lucidus* |
|---|---|---|
| Kauai | *H. (obscurus) procerus* | *H. lucidus hanapepe* |
| Oahu | *H. obscurus ellisianus* | *H. l. lucidus* |
| Molokai | (lacking) | (lacking) |
| Maui | (lacking) | *H. lucidus affinis* |
| Lanai | *H. obscurus lanaiensis* | (lacking) |
| Hawaii | *H. o. obscurus* | *H. (lucidus) wilsoni* |

*H. obscurus* is related to *Loxops virens* but is larger and has a much longer, more decurved bill and a relatively shorter tail. Its representative on Kauai (*procerus*) has a huge bill and is best treated as a distinct species. All members of the *obscurus* group, with the doubtful exception of *procerus,* are extinct. *Hemignathus obscurus* fed on insects which it sought by hitching along the trunks or larger limbs of trees as would a creeper or woodpecker, meanwhile probing in crevices in the bark with its bill in search of prey. At times it would tap or pry away a loose bit of bark with its thin, delicate bill. It also probed in flowers for nectar and insects and in the deep leaf axils of the climbing pandanus (*Freycinetia*) for insects.

In *H. lucidus* (all three races of which are probably extinct) the upper mandible is like that of *obscurus* but the lower is much shortened and thickened producing a unique bill. This species like *obscurus* crept along the trunks of trees in search of insects. It used its heavier lower mandible to pry or chip off bits of bark; the tapping of its bill when so doing was sometimes audible (Perkins, 1903: 428). The bill of *lucidus* was not well adapted to probing for nectar and this species rarely visited flowers.

In *H. wilsoni,* the geographical representative of the *lucidus* group on Hawaii, the lower mandible has become straight, heavy and like a chisel. It was always commoner than any race of *lucidus* and still occurs in fair numbers. Munro (1944: 119) wrote of it: "All its muscles are well developed, especially those of the head and neck, and its skull is exceptionally thick. The head is larger than that of the Kauai species [*H. lucidus hanapepe*] and we had difficulty in getting the skin over it. The mandible seems to extend further back than in most birds. I believe this has been brought about by its habit of using the lower mandible as a woodpecker does its bill. It uses great energy in beating at the bark and wood, breaking off pieces and dropping them. . . . It drives the lower mandible with considerable force into the crevices of the bark, the mouth kept open, the tapping noise being plainly heard at a distance. . . . When it gets the lower mandible inserted it uses it as a lever. . . . The pieces which break off it takes with both mandibles and throws off, sweeping the long upper one into the crevices opened on the branch." Perkins (1903: 428) adds: "Not infrequently it lays hold of a projecting piece of bark or the stump of some small broken branch, and shaking its head from side to side, and pulling in all directions, endeavours to tear it down. If unsuccessful in the attempt, it will alternate this treatment with a shower of blows from its gaping bill." Unlike its congeners, *H. wilsoni* never visits flowers for nectar.

The difference in the bills of *Hemignathus obscurus* and *H. wilsoni* is greater than that existing within the limits of many families of birds, much less within a single genus. Yet in other respects these two species are very similar indi-

cating close relationship, which is further shown by the intermediate species, *H. lucidus*. We may assume that the modification of the bill in *Hemignathus* has been rapid. Further evidence on this point is given by geology. The latest authority (Stearns, 1946) suggests that the Hawaiian Islands are of Pliocene and later age. Assuming it was mid-Pliocene before the islands were forest-clad and suitable for birds, about five million years would be available for the evolution of the Drepaniidae. Speciation within *Hemignathus*, one of the specialized later genera of the family, occupied, we may be sure, only a small portion of this time.

Thus in *Hemignathus* a rapid divergence in feeding habits and correlated structure of the bill has occurred. One species, *lucidus*, is intermediate and appears ill adapted for either the old or new habits. The three races of *lucidus* were uncommon and local and the total population of each may have been only a few hundred. This further suggests that the species was relatively unsuccessful. *H. wilsoni* was commoner and more generally distributed ecologically and since it occurred on the large island of Hawaii, its total population was probably several thousand. *H. o. obscurus* of Hawaii was originally as common as *wilsoni* or more so, suggesting that it too was well adapted to its particular ecological niche. All forms of *Hemignathus* are strictly limited to the forests and doubtless individuals were blown or carried from island to island only very rarely and accidentally. It is unlikely that the population of one island was subdivided into partially isolated colonies, unless temporarily on the larger islands by lava flows.

The rapid divergent evolution believed to have occurred in *Hemignathus* seems to represent what Simpson (1944: 206) has called quantum evolution, i.e., "the relatively rapid shift of a biotic population in disequilibrium to an equilibrium distinctly unlike an ancestral condition." While there is some objection to this use of the word "quantum," the type of evolution in question is real and of great importance, since it is often involved in the major transformations that give rise to families and other higher taxonomic categories. In quantum evolution preadaptation, selection, and sometimes non-adaptive genetic change, all play a part. The most plausible genetic basis for such evolution differs with the importance assigned to each of these factors (Simpson, 1944; Wright, 1945); probably the role of each varies under different circumstances. It seems likely that *Hemignathus obscurus* and *H. lucidus* were originally geographical representatives of a single species and quite similar morphologically at the time they were first brought into competition by secondary overlap in range. Selection then began and led to increasing divergence in feeding habits and bill structure, just as in the two Kauai species of *Loxops* but more pronounced. Perhaps the overlap in range occurred first on Hawaii, since *wilsoni* of that island is the most modified species. In *Hemignathus*, therefore, preadaptation and non-adaptive change may have been relatively unimportant and selection (in the presence of empty ecological niches) of great significance. If such were the case, it is not essential to postulate a number of very small, partially isolated populations, as considered necessary by Wright (1945) if both selection and non-adaptive genetic drift are to be simultaneously effective.

The changes in the bill of *Hemignathus* illustrate the transformation of a specialized structure. The result, as seen in *H. wilsoni*, is somewhat grotesque but appears effective and may be further perfected. Equally peculiar bills occur in a few continental birds of wide distribution (and hence successful) such as crossbills (*Loxia*), skimmers (*Rynchops*), or certain parrots. The evolutionary modification of a specialized structure, though rare, may be of great importance occasionally (Amadon, 1943).

Transformation of the bill has occurred

at least four times in the Drepaniidae, although *Hemignathus* is the only genus in which the intermediate form still existed. In the peculiar *Pseudonestor xanthophrys* of Maui both the upper and lower mandibles are very heavy. Its bill is used to crush dead twigs of the koa tree thus exposing larvae of cerambycid beetles. *Pseudonestor* is closely allied to *Hemignathus;* perhaps these two came into competition at a time when they were more similar, leading to divergence. In the five species of the genus *Psittirostra* the bills are, to varying degrees, finch-like. The genus probably evolved from a less specialized ancestor, similar to *Loxops virens*. The extreme of this genus is *Psittirostra kona* which cracked hard seeds in its huge bill. The little known *Ciridops* also evolved a finch-like bill.

## Summary

The Hawaiian honey-creepers (Drepaniidae) are an excellent example of rapid adaptive radiation occurring in recent geological periods in a favorable environment having many vacant ecological niches. In the genus *Hemignathus* three species, one of them intermediate and apparently ill adapted, represent stages in a rapid ecological shift in habits and correlated morphological structures of the type Simpson has called quantum evolution. Such evolution, though important, occurs rarely or locally and is comparatively rapid, so that direct evidence, fossil or recent, is scanty. *Hemignathus* provides perhaps the only known contemporary example among birds and even here some of the species involved are extinct. Simpler examples of divergence in the Hawaiian genera *Phaeornis* and *Loxops* suggest that much of the impetus for such change comes from competition between similar species.

I am indebted to Drs. Ernst Mayr, John A. Moore and Bobb Schaeffer for valuable suggestions.

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# NATURAL HYBRIDIZATION OF *SALVIA APIANA* AND *S. MELLIFERA*

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Reference is made with increasing frequency in plant taxonomic literature to the occurrence in nature of interspecific hybrids: *Ceanothus* (McMinn, 1942), *Salvia* (Epling, 1942), *Balsamorhiza* (Ownbey and Weber, 1943), *Clematis* (Erickson, 1945), *Wyethia* (Weber, 1946), and *Aquilegia* (Munz, 1946), to cite some of the more recent. In these examples, so far as the reader is able to judge, $F_1$ hybrids, backcrosses and even hybrid swarms may occur.

Because of the weak intrinsic barriers between species which characterize these and many other plant groups, the assumption might logically be made that gene flow between the species concerned would obliterate or blur the specific morphological differences if long continued. Yet the taxonomic treatment in such papers suggests that the constancy of the species concerned has not been materially affected.

The observations which follow seem to confirm this latter view and to indicate that gene flow from species to species does not ordinarily occur, or if so, at a level difficult to distinguish from expected intraspecific variation, even though continued hybridization may seemingly provide a channel.

## DISTRIBUTION OF THE SPECIES

*S. mellifera* and *S. apiana* are two widespread, characteristic, and abundant members of the coastal sage community of California (Epling and Lewis, 1942). This community is developed chiefly on the coastal plain of Southern and Baja California as far as the Viscaino desert, and at lower elevations in the mountains which border it, but extends northward at lower elevations in the South Coast Ranges as far as the Bay Region of central California. The ranges of the species referred to are largely coterminous with the area indicated, but *S. apiana* extends into the chaparral and, in the interior, reaches the Mohave and Colorado deserts. *S. mellifera* is more coastal. The fact that these are perennial subshrubs permits observation of the same individuals over a period of years.

As indicated on figure 1, these two species are sympatric over a large area, from Santa Barbara County to San Diego County. Within this area they are frequently in contact, sometimes as individuals, but more often as connecting colonies which may occupy many acres. Such colonies may be relatively pure in stand, or may be associated with other members of the community. When in contact, the colonies tend to border each other, or one may be enclosed by the other, *S. apiana* usually occurring on the drier sites.

There is no reason to believe that this intimate association is, in general, recent and induced by disturbance. On the contrary, the extensive area occupied in common, and the present spatial relationships and associates, indicate that the species have been in contact for a long period, although disturbance, particularly burning, has undoubtedly produced a closer or more recent contact in many places.

## HYBRIDIZATION

Wherever two species of *Salvia* (sect. *Audibertia*) occur together hybrids are

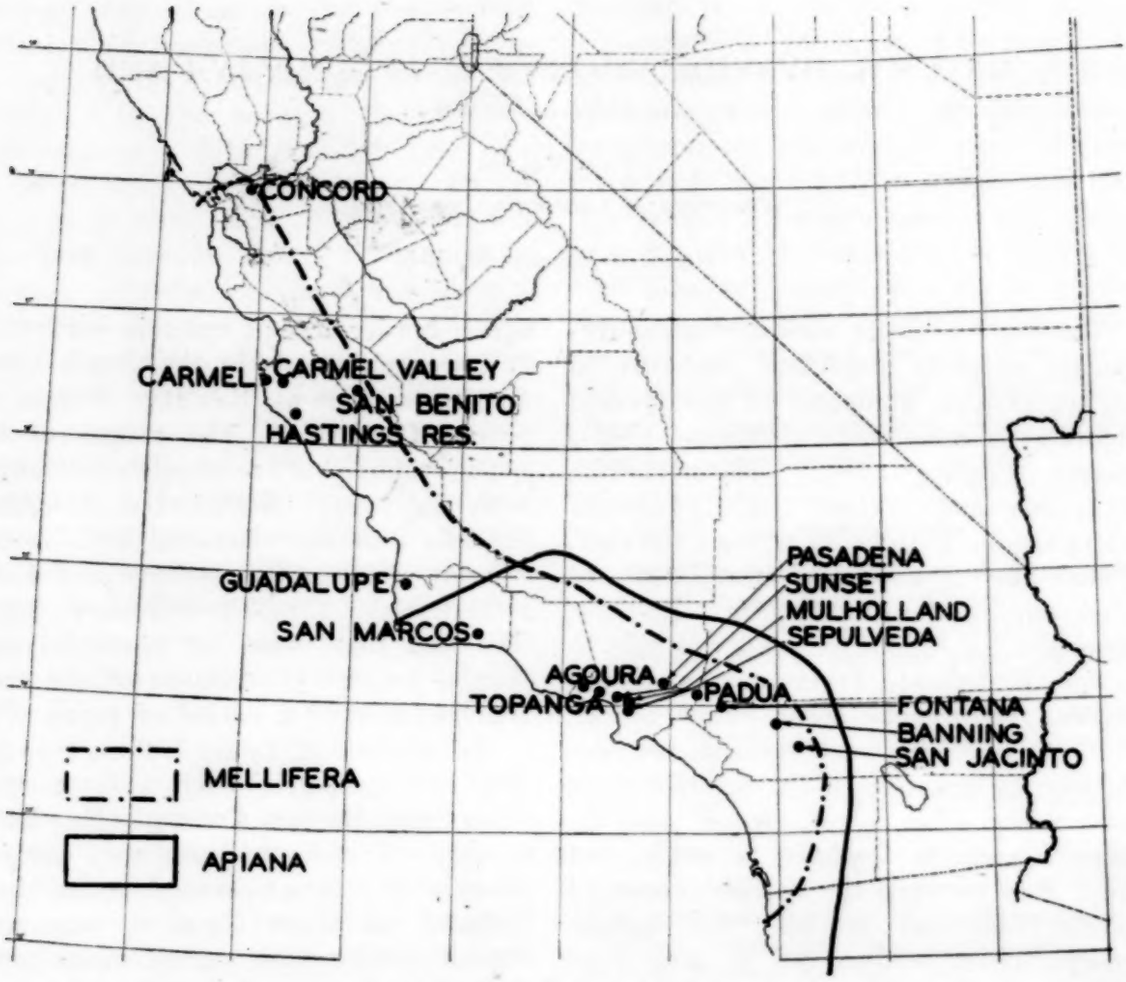


FIG. 1. A map of California showing the range limits of *S. apiana* and *S. mellifera* and the location of the colonies referred to in table I, in figures 3 and 4, and in the text.

often observed. This fact is not unexpected, since all of the species referred to in this paper have the same chromosome number ($n = 16$), and similar karyotypes. A list of known wild interspecific hybrids will be found in Epling (1942). Furthermore, allopatric species, such as *S. leucophylla* and *S. Clevelandii,* hybridize freely when grown together (records of the Rancho Santa Ana Botanic Garden). This tendency is particularly noticeable in *S. apiana* and *S. mellifera,* perhaps because they range widely and are more frequently in contact.

The hybrids usually observed are probable $F_1$ individuals. As indicated in figures 2, 3, and 4, they are morphologically intermediate between the two parents. Once known, they are readily discerned in passing, even at a distance, because of differences in habit, particularly of the inflorescence. Since the hybrids usually occur intermixed with *S. mellifera,* even though *S. apiana* may be nearby, it would appear that *S. mellifera* is usually the female parent.

But close inspection of a population in the vicinity of such hybrids sometimes discloses *mellifera*-like individuals, some characters of which, particularly the conformation of the corolla and the number of terminal whorls, definitely vary toward *S. apiana.* Such individuals are sufficiently like *S. mellifera* to pass casually as such in the herbarium, or even in the field. Upon more careful analysis still other individuals may be found, which, although scarcely distinguishable from

*S. mellifera,* still vary in the direction of *S. apiana* to a slight but detectable extent. It is evident, therefore, that in such populations selfing or backcrossing, or both, are in progress. The fact that the plants in question are variously intermediate between the putative $F_1$ plants and *S. mellifera* indicates backcrossing.

The fertility of the $F_1$ plants is very low. In nature, after open pollination, about 50 per cent of the nutlets develop, but most of the embryos within shrivel and fail to mature. Those which are viable would be, at most, approximately 2 per cent of the total and probably less. But the offspring is vigorous, both in nature and in the garden. After open pollination, again, only about 50 per cent of the nutlets of these plants develop, but the proportion of viable seeds may be as high as 30 per cent to 40 per cent, although probably less.

## Morphology

Among the contrasted characters which distinguish the species, the two which are most definitive and show the greatest differences are the conformation of the corolla, particularly with respect to the proportions of the upper and lower lips and the position of the stamens and style, and the number of terminal whorls in the inflorescence. These have been accordingly employed as the chief criteria for the detection of hybrids.

By reference to figure 2 it will be observed that, relative to the lower lip, the upper lip of *S. mellifera* is long and erect. At first more or less plane, it becomes somewhat recurved laterally. The upper lip of *S. apiana,* on the other hand, is actually and relatively much shorter. It is generally recurved in the upper half and is commonly obscured by the remarkable folding of the lower lip.

The stamens of *S. mellifera* are seated in the throat, are appressed to the upper lip, and the anthers are connivent and about equal to the lip or slightly exserted beyond it. The style rests between and behind them. The stamens of *S. apiana* are seated on the lower lip, are erect, and

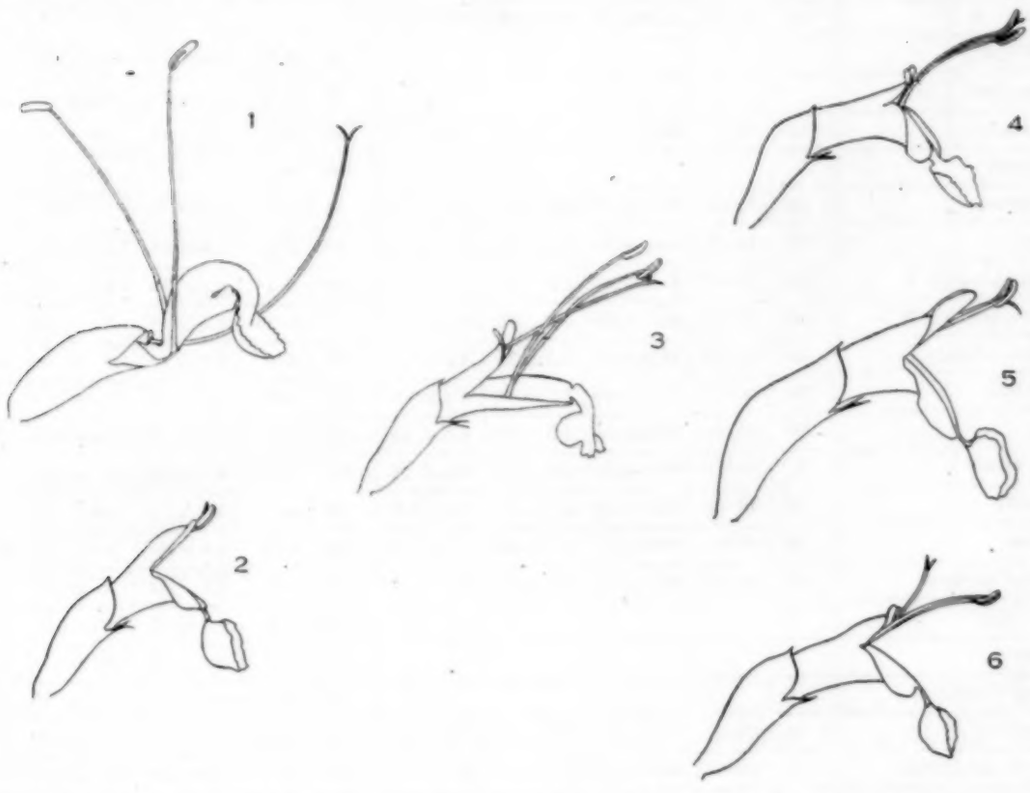


Fig. 2. Drawings of flowers. 1. *S. apiana,* 2. *S. mellifera,* 3. $F_1$, 4–6. Backcrosses.

are somewhat arcuately divergent. The style is strongly deflexed to one side of the corolla.

With these characters in mind, a word may be said concerning pollination. The observed visitors are principally bees, especially honeybees. *Bombus* is frequently seen. The conformation of the flower of *S. apiana* is such that pollination under any circumstances is hard to visualize, because of the divergent stamens, and the disassociated style which is often deflexed. Even contact with the body hairs of any but the larger bees would seem improbable or infrequent. Nevertheless, *S. apiana* does set seed, and bagging experiments indicate that it is chiefly cross-pollinated. Furthermore, the frequency of $F_1$ hybrids in nature suggests that the two species are frequently cross-pollinated. But the fact that these hybrids almost always occur with *S. mellifera,* indicates that such crossing proceeds usually in one direction only. The conformation of the two flowers may therefore constitute a partial barrier between the species. Because of the greater similarity of the $F_1$ to *S. mellifera,* this barrier is apparently much less pronounced between the hybrids and that species, which may account for the abundance of backcrosses under some conditions. I have observed only one individual which might be construed as a backcross to *S. apiana.*

Though the vegetative habit of the two species is sharply different, it is difficult to treat quantitatively. The vegetative

TABLE I. *Geographic distribution of corolla ratio and whorl number of the colonies of* S. mellifera *examined.* R = Range of ratios, M = Mean, $\sigma$ = Standard deviation, V = Coefficient of variability.

| Localities | Upper lip/lower lip | | | | | Whorls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | R | M | $\sigma$ | V | N | R | M | $\sigma$ | V |
| Concord | | | | | | 50 | 3–8 | 5.5 ± .14 | .99 ± .10 | 18 ± 1.8 |
| Carmel | 21 | 53–70 | 60.9 ± 1.0 | 4.9 ± .76 | 8.1 ± 1.2 | | | | | |
| Carmel Valley | | | | | | 50 | 3–7 | 4.8 ± .12 | .86 ± .09 | 18 ± 1.8 |
| Hastings Res. | | | | | | 50 | 3–7 | 4.6 ± .14 | .98 ± 9.8 | 21 ± 2.0 |
| San Benito | 50 | 45–71 | 56.5 ± .75 | 5.3 ± .53 | 9.4 ± .9 | 50 | 3–7 | 4.2 ± .12 | .86 ± .09 | 20 ± 2.0 |
| Guadalupe | | | | | | 200 | 3–8 | 5.4 ± .07 | .94 ± .05 | 17 ± 0.8 |
| San Marcos | 50 | 52–71 | 59.5 ± .67 | 4.7 ± .47 | 7.9 ± .8 | 50 | 4–7 | 5.4 ± .10 | .71 ± .07 | 13 ± 1.3 |
| Agoura | 50 | 52–74 | 60.3 ± .72 | 5.1 ± .51 | 8.4 ± .8 | 50 | 3–6 | 4.6 ± .11 | .79 ± .08 | 17 ± 1.7 |
| Topanga progeny T | 139 | 38–73 | 57.2 ± .46 | 5.4 ± .32 | 9.5 ± .6 | 138 | 4–11 | 6.2 ± .10 | 1.2 ± .07 | 19 ± 1.1 |
| Topanga progeny N | 76 | 38–73 | 56.1 ± .65 | 5.7 ± .48 | 10.2 ± .8 | 75 | 5–11 | 6.5 ± .16 | 1.4 ± .11 | 21 ± 1.7 |
| Topanga progeny F | 63 | 47–69 | 58.3 ± .66 | 5.2 ± .46 | 8.9 ± .8 | 63 | 4–9 | 5.9 ± .12 | 1.0 ± .09 | 18 ± 1.6 |
| Mulholland | 50 | 46–72 | 57.7 ± .69 | 4.9 ± .49 | 8.4 ± .8 | 50 | 4–9 | 6.1 ± .15 | 1.1 ± .11 | 18 ± .8 |
| Sunset | 50 | 51–75 | 61.8 ± .74 | 5.2 ± .52 | 8.6 ± .9 | 50 | 3–7 | 5.5 ± .13 | .97 ± .10 | 18 ± 1.8 |
| Sunset mixed | 203 | 46–77 | 59.6 ± .39 | 5.6 ± .28 | 9.4 ± .5 | 106 | 3–10 | 6.4 ± .14 | 1.5 ± .10 | 23 ± 1.6 |
| Sepulveda | 50 | 48–76 | 58.1 ± .80 | 5.7 ± .57 | 9.8 ± 1.0 | 50 | 4–8 | 5.4 ± .13 | .95 ± .10 | 18 ± 1.8 |
| Pasadena | 50 | 49–71 | 59.5 ± .68 | 4.8 ± .48 | 8.1 ± .8 | 100 | 4–8 | 5.6 ± .08 | .81 ± .06 | 15 ± 1.1 |
| Pasadena progeny | 49 | 48–73 | 60.3 ± .75 | 5.3 ± .53 | 8.7 ± .9 | 50 | 4–9 | 6.4 ± .15 | 1.1 ± .11 | 18 ± 1.8 |
| Padua Hills | | | | | | 50 | 3–9 | 6.1 ± .17 | 1.2 ± 1.2 | 20 ± 2.0 |
| Fontana | | | | | | 31 | 3–7 | 4.9 ± .17 | .94 ± .12 | 19 ± 2.4 |
| Banning Road | | | | | | 50 | 3–7 | 5.0 ± .14 | 1.0 ± .10 | 21 ± 2.1 |
| San Jacinto | 30 | 49–67 | 56.7 ± .95 | 5.2 ± .67 | 9.1 ± 1.2 | 50 | 3–7 | 4.5 ± .13 | .92 ± .09 | 21 ± 2.1 |
| Sunset $F_1$ progeny | 25 | 41–63 | 50.6 ± .99 | 5.1 ± .72 | 10.0 ± 1.4 | 25 | 5–11 | 7.9 ± .30 | 1.5 ± .21 | 19 ± 2.6 |
| Salvia apiana | 20 | 10–17 | 12.8 ± .40 | 1.8 ± .29 | 14.0 ± 2.2 | 50 | 6–16 | 10.6 ± .29 | 2.1 ± .21 | 20 ± 2.0 |

part of *S. apiana* is relatively low and rounded. From it arise each year elongate flowering shoots which later die back to the base. The number of vegetative internodes of these shoots is considerably more than of similar shoots of *S. mellifera*. The number of branches (and whorls) which will bear flowers is considerably higher. The comparable shoots of *S. mellifera* are often unbranched, but typically produce one, or sometimes two pairs of branches below the terminal whorls. The internode patterns of the two species differ considerably in the mean. But they are affected greatly by the vigor of the plant and the conditions of growth (and by lodging) and the differences, particularly in respect of branching, are thus often obscured. The number of terminal whorls in a spike appear to be less subject to such factors, and is more readily determined. Hence, this character has been employed here as an index to other observed differences in the inflorescence.

## Geographic Variation

The two character-complexes described above, one vegetative, the other floral, were chosen for study because they, more than any others, seem to provide the contrast needed whereby the effects of hybridization might be studied and expressed. Both are mensurable. But the study of other characters was not neglected. Their pattern of variation conforms to that of the criteria chosen. The basic data recorded for these criteria, including their standard errors, will be found in table I. The means of the corolla proportions are shown graphically in figure 3 for a series of colonies from the

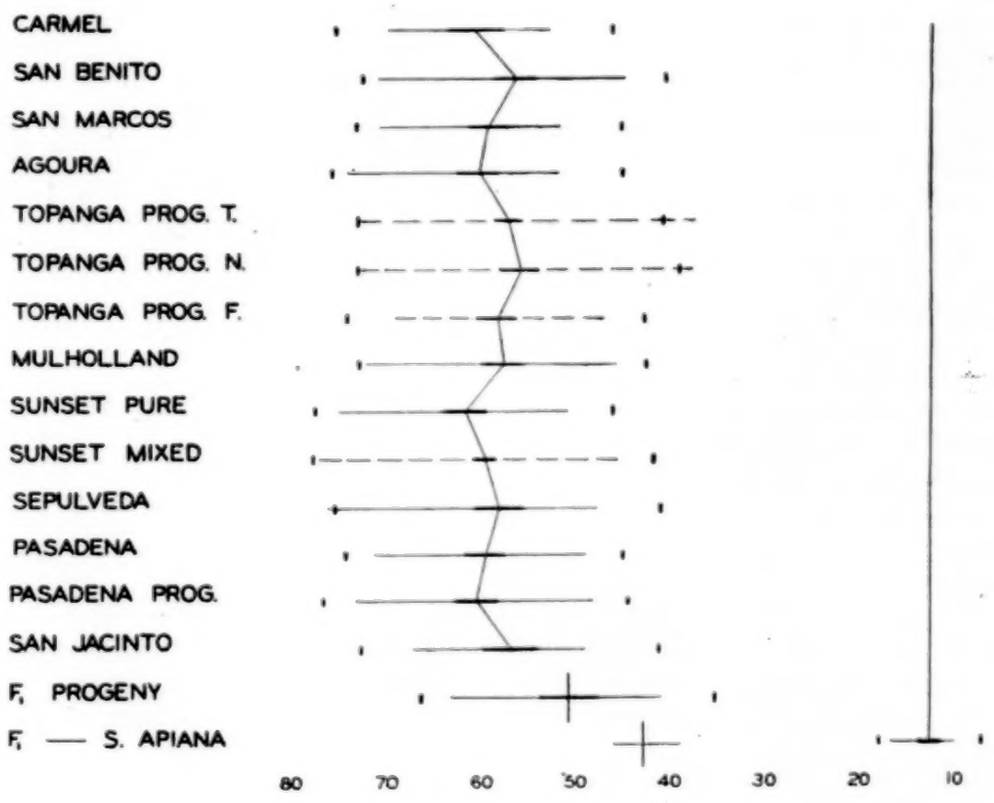


Fig. 3. Ratio of length of upper lip to lower lip of corolla, by colonies, geographically arranged. The observed range (horizontal line), its theoretical limits (vertical bar $\pm 3\sigma$), the mean (connected by vertical line) and its theoretical limits (horizontal bar $\pm 3\sigma$) are shown for each colony. Broken lines indicate colonies in which hybrids were found. Below, the same for *S. apiana* (one colony), 3 $F_1$ plants (Sunset mixed colony) and the garden grown progeny of one.

Bay Region to the San Jacinto Mountains (figure 1), that is, from a point far beyond the range and influence of *S. apiana,* to the center of its concentration. Similar data for the number of whorls of the inflorescence are shown graphically in figure 4. The samples were obtained at random along transects.

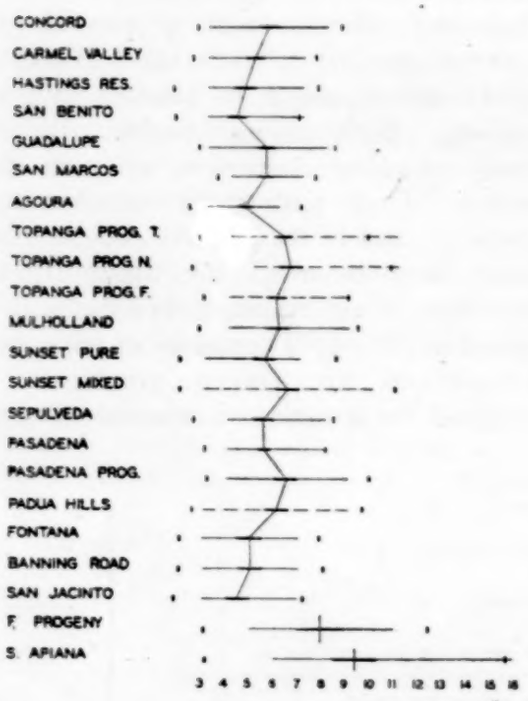


FIG. 4. Whorl number, by colonies, geographically arranged. The observed range, its theoretical limits ($\pm 3\sigma$), the mean (connected by vertical line) and its theoretical limits ($\pm 3\sigma$) are shown for each colony. Broken lines indicate colonies in which hybrids were found. Below, the same for *S. apiana* (one colony) and the garden grown progeny of an $F_1$ plant (Sunset mixed colony).

The extent to which hybridization may occur can be illustrated by a mixed colony found on Sunset Boulevard, Westwood, which exists on a spur running down from the Santa Monica Mountains. The vegetation of the spur is that characteristic of the region, but the structure of the community has been modified by burning and contiguity to inhabited areas. The colony consists largely of *S. mellifera* intermixed with *Artemisia californica,* some *Rhus integrifolia* being present. *S. apiana* is located chiefly on the abrupt slopes of a road-cut but in recent years has extended its area somewhat onto the surface of the spur. Hence, although both species are doubtless normal constituents of the vegetation, their intermixture here and subsequent hybridization is probably the result of disturbance. Gradual replacement of individuals of both species is indicated by differences in size, and hence presumably of age.

For convenience of reference, 276 individuals in the immediate vicinity of *S. apiana* were marked with metal tags. Three of these were obviously $F_1$s, and 14 were construed as backcrosses or possibly as segregates from selfing. The remaining individuals were either typical *mellifera* or varied somewhat in the direction of *S. apiana* as follows: the stamens might be more exserted or wider set and spreading, or the whole plant might show greater vigor, with more internodes and larger flowers which might be fewer in number per whorl, or the upper lip of the corolla, instead of being laterally recurved, might be somewhat recurved toward the tip, or, as pointed out below, the flowering period might be later. Such plants were interpreted as probable second backcrosses. Their number was indeterminate because of the difficulty of distinguishing them from variants of *S. mellifera.*

Greater exsertion of the stamens beyond the upper lip, for example, might indicate the influence of *S. apiana.* The range of exsertion in this colony, exclusive of the plants definitely hybrid, was from 0 or less to 40, the mean being 14 (measured in divisions of a micrometer eyepiece). The proportion of stamens shorter than the upper lip was about 18 per cent. The range of a colony of *S. mellifera* at Carmel, outside the range of *S. apiana,* was from 0 or less to 32, the mean being 15. The proportion of stamens shorter than the upper lip was about 10 per cent. The extremes of the Sunset colony might therefore be interpreted as caused by *apiana* genes. But the smaller mean and the greater proportion of in-

cluded stamens would suggest simply intercolonial variation. In other words, if infiltration of genes is actually taking place from *S. apiana* into *S. mellifera,* its effect is not certainly distinguishable from what may be normal variation within the species *S. mellifera.*

If the species have been in contact for a long period and if hybridization has taken place during this time, both of which seem probable, then one might expect the populations of *S. mellifera* in contact with *S. apiana* to show a morphological trend toward it, or to be more variable than those outside its range. Neither is true of the two strongly contrasted characters (nor, so far as I can determine, for any others) of *mellifera* colonies which are not now immediately in contact with and hybridizing with *S. apiana.*

The range and mean of corolla proportions of the San Benito colony, for example, 100 miles north of the range of *S. apiana,* are not significantly different from the colony obtained in the San Jacinto Mountains or from a colony collected in the Santa Monica Mountains on Mulholland Drive, which lies within perhaps five miles of the mixed colony on Sunset Boulevard. Furthermore, the mean and range of a pure colony on Sunset Boulevard, two-tenths of a mile from the hybridizing colony, is even less like *S. apiana* than the San Benito colony.

Comparison of the means and ranges of whorl number gives, on the whole, similar results. In this case, however, the colonies of *S. mellifera* found in Los Angeles County do show a consistent trend toward *S. apiana.* But this trend is not necessarily the result of *S. apiana* influence but may be a response to local conditions. As a test of the degree to which whorl number may be influenced by local conditions, progeny of a pure colony found at Pasadena which showed no trend toward *apiana* were grown at Westwood with irrigation. Its (Pasadena) mean whorl number under these conditions increased from 5.6 to 6.4.

The flowering periods of the two species and the hybrids were also compared. *S. mellifera* comes into flower perhaps a month earlier than *S. apiana* and they flower together for perhaps a month. The exact times and overlap depend upon the locality and season. In any given colony of pure *S. mellifera* the individuals begin to flower within a short period, usually within about ten days or two weeks. A record was kept of the approximate date of first flowering of the tagged individuals in the mixed colony on Sunset Boulevard. The $F_1$ plants were more or less intermediate between the species, verging somewhat toward *S. apiana.* The backcrosses were generally later than *S. mellifera,* But occasional individuals, not otherwise distinguishable from *S. mellifera,* came into flower as late as the $F_1$ plants or even *S. apiana.* Pure colonies of *S. mellifera* do not generally show such laggards and in the case of the pure colony on Sunset Boulevard, none were found.

The San Marcos colony is also instructive. This colony does not differ significantly in corolla proportions from that in San Benito County, nor in whorl number from the Concord colony. Most of the plants measured were two or three year old plants which had come into a burn. Adjacent and included in the count were a few old *mellifera* plants; nearby were old *apiana* plants. No evidence exists, to be sure, that hybridization actually took place before the burn but in view of the close association of both species and the usual occurrence of hybrids under these conditions, past hybridization would normally be expected. Yet in the new generation there is no evidence of *apiana* influence.

The fact that *apiana* influence is confined to mixed colonies suggests that relatively short distances may be a barrier to the transfer of pollen and seeds. The two pure colonies on Sunset Boulevard (Sunset pure and Sepulveda) are about $\frac{3}{10}$ mile on either side of the mixed colony. Perhaps no more than a coin-

cidence (although the habits of pollinators might make it more than a coincidence) this distance seems also to be a limiting factor in hybridization between two races of *Delphinium Hanseni* (Epling and Lewis, unpubl.). As a test of the distance over which pollen might be expected to travel, progenies were grown from each of ten wild plants of *S. mellifera* taken along a transect of approximately 100 yards in a mixed colony in Topanga Canyon, near Santa Monica, leading away from a stand of *S. apiana.* The differences in range, mean and variability between the progeny of the further five (F) and the nearer five plants (N) will be found in table I and figures 3 and 4. The immediate effect of hybridization seems to have a relatively short radius.

The facts thus far obtained argue, I believe, that the effects of hybridization of *S. apiana* and *S. mellifera* are localized and that gene exchange which would otherwise blur the specific lines is blocked although hybridization may be continual when and where the species are in contact. Still another fact bears out this conclusion. *S. apiana* is known to hybridize not only with *S. mellifera,* but also, in different parts of its range, with *S. leucophylla, S. Clevelandii, S. Munzii, S. eremostachya* and *S. Vaseyi.* These species are more than usually diverse and well defined. There is no evidence that any of them has been affected by hybridization except for a possible contamination of *S. apiana* by *S. Vaseyi.*

## Discussion

The appearance of these hybrids may be the result of man made disturbance. Yet this seems not always to be the case, and in any event, the natural disturbances which occur in any community might be expected to provide occasional opportunity for intermixture and hybridization if the species are compatible. Given disturbance, the amplitude of tolerance of *S. apiana* and *S. mellifera* and their compatibility is sufficient to allow germination and establishment of many individuals of both species and their hybrids side by side. Nevertheless, the channel of gene exchange by which the two species might coalesce appears to be blocked, almost at its inception.

This effect is probably the result of a combination of isolating mechanisms operating in a community in which the norm of succession is not materially modified. The production of $F_1$ individuals is first limited by distance, colonial habit and ecological preference, by structural differences of the flower in relation to the pollinators, and by partial seasonal isolation. Given local disturbance, hybrids may appear. The low fertility of the $F_1$ suggests the loss of incompatible genetic factors which are further eliminated in the backcrosses. But the continued partial sterility of the backcrosses suggests that a balanced genotype is still not achieved and that further recombinations which might produce it would still be relatively few. Even though successive backcrosses become more melliferoid, they might find establishment difficult in competition with parental types already selected for that particular environment, although such competition would probably be less severe in a disturbed habitat. But the hybrids would be competing with a large number of the parental species by limitations on their pollen and seed dispersal. Not only would the abundance of parental pollen be much greater than that of the hybrids, but the greater abundance of parental seeds would ensure a greater number of survivals from chance loss, as by seed eaters.

Being thus localized in a disturbed area, succession might then be expected to eliminate the hybrid elements and restore the community to its previous condition. Observations such as those of Piemiesel and Lawson (1937) indicate that after relaxation or removal of the disturbing factors regeneration of the original vegetation may proceed rapidly even in regions much modified.

But should disturbance of the community proceed to the point where the successional equilibrium is lost, as may be the case in densely peopled areas, hybridization might then become so prevalent that the two species would show "contamination" to the degree ascribed to *Tradescantia occidentalis-canaliculata* (Anderson and Hubricht, 1938), to *Iris* species (Riley, 1938), or even to the extent of hybridization suspected in *Teucrium canadense* (McClintock and Epling, 1946). This would depend upon the relation of the external isolating mechanisms to the environment and the degree to which they were integrated with the genetic systems involved. The spread of genes through a population under these conditions of disturbance might also be a direct consequence of transport by man.

Given stabilization of a new successional pattern and hence of a new pattern of competition, as may result from a combination of edaphic and climatic changes, then a hybrid combination might emerge as a new specific entity. There is evidence to suggest that certain species of *Delphinium* (Lewis and Epling, unpubl.) may have arisen in this way.

The phenomenon which these species of *Salvia* typify is apparently widespread amongst flowering plants. Many closely related species of plants are able to occupy common territory despite a relatively high degree of compatibility. This may also be true of certain animals, although in these cases the extrinsic barriers would appear not to be ecological (see, for example, Diver, 1940, p. 326, and Dice, 1940. However, Heuts (1947) has described a carefully investigated situation in *Gasterosteus aculeatus* in which gene flow is apparently preserved between two populations which are ecologically isolated). Furthermore, there is evidence to suggest that such compatibilities may persist for long periods. The London Plane, a well known hybrid between *Platanus orientalis* and *P. occidentalis,* is frequently cited. To judge from their present distribution in Asia Minor and the eastern United States, the parent species have probably been separated since the Miocene. These, to be sure, are allopatric. Other evidence suggests that several species of *Quercus* may have been in contact in the eastern United States at least since the Pleistocene and, although hybridizing under some conditions, have preserved their identity (Stebbins, Matzke and Epling, 1947; see also Dobzhansky, 1941, p. 259; Muller, 1938; and Salisbury, 1916, 1918). If this is true, Dobzhansky's hypothesis (1940) might lead one to expect a more complete isolation to have arisen during this period, if a premium is put upon complete isolation. Yet such seems not to be the case. The balance between extrinsic and intrinsic barriers of *Quercus* seems to have been sufficient under the circumstances of the major climax to preserve for a long period the adaptive peaks which each species represents. Hence, it may be that in plants the development of complete intrinsic barriers may often be purely fortuitous.

Indeed, the plasticity conferred by partial reliance upon ecological barriers and hence the opportunity of hybridizing may be advantageous. For, with a change in the environment which exceeds the tolerance of two given sympatric species, an adaptive peak suited to the new environment might be realized from a hybrid combination. Such a process may have been of particular advantage to plants in a region undergoing climatic change, as that which has apparently occurred in the temperate zone during the Tertiary. Although the trend of change may have been gradual, the modification of climatic control by topographic and edaphic factors has undoubtedly created a multiplicity of habitats, which may on occasion have been relatively abrupt. In other words, there seems no reason why Wright's concept of the impact of selection may not be extended to plant species which are still capable of some gene interchange. The force of this argument will

rest upon what we may be able to learn about the relative effect of ecological requirements upon gene flow.

## Summary

The effects of natural hybridization of *Salvia mellifera* and *S. apiana* are described. Despite compatibility sufficient to permit frequent localized hybrid swarms, it appears that the constancy of the species is maintained. This is ascribed to a combination of isolating factors operating in a community in which the norm of succession, and hence the equilibrium of competition, is not materially modified. The potentiality of gene flow thus preserved might prove advantageous and provide a genotype which could exploit an abrupt change in environment.

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# HYBRIDIZATION IN A POPULATION OF QUERCUS MARILANDICA AND QUERCUS ILICIFOLIA

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Based on observations of comparative external morphology, hybrids have been described involving most of the species of *Quercus* found in the eastern United States (Trelease, 1917). These occur mostly as single trees or small groups in company with their putative parents. No careful studies have yet been made to see whether these hybrids, either by backcrossing or segregation, have affected the variability of the populations of their parental species. MacDougal (1907) showed that both *Q. borealis* var. *maxima* × *phellos* (*Q. heterophylla* Michx.) and *Q. marilandica* × *phellos* (*Q. Rudkini* Britton) produced many vigorous seedlings from acorns gathered from wild trees. Those of the former hybrid showed strong segregation for the characteristics of the parental species, while those of the latter were relatively constant.

Oaks would therefore seem to be favorable material on which to make studies of the effects of hybridization on variability in species populations. Two species particularly well suited to such studies are *Quercus marilandica* Muench., the black jack oak, and *Q. ilicifolia* Wang., the black scrub oak, which occur together over a large area of the coastal plain in Long Island, New York, and the pine barren region of New Jersey. The two species are very abundant, and for the most part can be distinguished at a glance. In the rest of their natural ranges, *Q. ilicifolia* and *Q. marilandica* are mostly well separated from each other either geographically or ecologically. *Q. ilicifolia* is frequent on the sterile hills of southern New England and southeastern New York, and extends southward in the Piedmont and Appalachian regions to North Carolina, and west to Ohio. *Q. marilandica,* on the other hand, reaches its northeastern limit on Long Island, and extends from there southward on the coastal plain to Georgia, thence west to Texas and Oklahoma, and northward in the Mississippi Basin to Ohio, Minnesota, and Nebraska. The range of *Q. marilandica* is therefore mostly southward and westward of that of *Q. ilicifolia.* Hybrids between the two species have been reported by Davis (1892) from Staten Island, New York.

The possibility of hybridization between these two species was first suggested to the senior author by a collection of leaves made by Mr. M. R. Birdsey, a graduate student in botany at Columbia University. Following this suggestion, the authors led an advanced botany class of this University on a field trip to the pine barren area one mile south of Lakehurst, Ocean County, New Jersey. In this region *Q. marilandica* was the most common species of oak, growing in an open stand among scattered trees of *Pinus rigida* on a flat plain of the Cohansey sand formation. Scattered among the black jack oaks were shrubs of *Q. ilicifolia,* and one half mile southeast of the first collecting locality, on a low knoll overlooking a cranberry bog, was a dense stand of *Q. ilicifolia.* From these two localities the class and the authors collected a population sample ("mass collection") representing 86 individuals of both species and possible inter-

mediates. Each individual was represented by a specimen consisting of one or more typical mature leaves, one or more twigs from the upper part of the tree or shrub, and several acorn cups, gathered either on the plant or immediately under it.

For comparison, a similar collection was made of *Q. marilandica* from Cliffwood, Monmouth County, N. J. Here the black jack oaks grow in a mixed deciduous forest, along with *Q. velutina, Q. phellos, Q. alba,* and *Q. montana,* on the Magothy sand and clay formation, near Raritan Bay. No shrubs of *Q. ilicifolia* were seen in the vicinity. Here 23 specimens were collected, but acorn cups could be found on only 14 of them.

Measurements of the specimens were made by members of the class and by the writers. Variations due to personal differences in measuring were avoided by having one person responsible for measuring one particular character on all of the specimens. Measurements of the standard series were made by the authors, who also checked the values for all specimens of an intermediate character.

In order to decide which characters to measure, a comparison was made between a series of specimens of the two species collected in various parts of their ranges, but in general outside of the region of overlap. These "standard" specimens, all in the herbarium of the New York Botanical Garden, are as follows:

*Q. ilicifolia:* Hinsdale, N. H., *Batchelder* in 1919; Andover, N. H., *Jack 3843;* Congamond Pond, Mass., *Seymour 270;* Martha's Vineyard, Mass., *Bicknell 3501, 3524, Brown and Seymour 1461,* and *Blodgett* in 1893; Pleasant Mills, N. J., *Morong* in 1873; Hackensack, N. J., *Halsey* s.n.; Huntingdon County, Pa., *Porter* in 1886; Centre County, Pa., *Mathias 1155, 1156, 1159;* Oil City, Pa., *Kuntze* in 1874; Rockingham County, Va., *Heller 799,* and *Heller* in 1893; Page County, Va., *Palmer and King* in 1901; Blue Ridge Mts., Va., *Bartley and Pontius 545;* Montgomery, Va., *Gibbes,* s.n.

*Q. marilandica:* Lancaster County, Pa., *Small* in 1889; Riverdale, Md., *House 541;* Williamsburg, Va., *Grimes 3226;* North Carolina, *Buckley* s.n.; Andersonville, S. C., *Gibbes* in 1865; De Kalb County, Ga., *Small* in 1894; Huntsville, Ala., *Baker* in 1897; Biloxi, Miss., *Lloyd and Tracy* in 1900; Cabell County, W. Va., *Gilbert 111, 811;* Scioto County, Ohio, *Camp 644, 645, 646;* Harrison County, Ind., *Deam 24369A;* St. Louis, Mo., *Riehl 235;* Levy, Ark., *Demaree 8872;* Amity, Ark., *Demaree 10021;* Texarkana, Ark., *Demaree 13469;* Benton, La., *Correll 10260;* Pottawatomie County, Kansas, *Hitchcock* in 1892.

Based on comparison between these series of specimens, the following characters were found to be most distinct in the two species:

1. *Leaf width.* The leaves of *Q. marilandica* are both larger and broader in relation to their length than those of *Q. ilicifolia.* The maximum width of the leaves is therefore the measurement which is most different in the two species.

2. *Number of veins on lower part of leaf.* The leaves of *Q. marilandica* have an elongate basal portion not found in those of *Q. ilicifolia.* This difference is best measured by counting the number of primary veins which depart from one side of the midrib below the large pair of veins which extends to the principal lobes. Although there is considerable overlap, the number of these veins is mostly higher in *Q. marilandica* than in *Q. ilicifolia.*

3. *Character of leaf base.* The leaves of *Q. ilicifolia* are cuneate at the base, while those of *Q. marilandica* are usually truncate or cordate. For convenience in scoring, three types of leaf base were recognized, as follows: 1. Leaves cordate, with the two sides forming a basal angle of more than 180 degrees. 2. Leaves truncate or somewhat cuneate, basal angle 90 to 180 degrees. 3. Leaves strongly cuneate, basal angle less than 90 degrees.

4. *Pubescence of under surface of leaves.* In *Q. marilandica* the mature leaves have tufts of brown trichomes in the axils of the veins, and elsewhere are smooth or bear very scattered stellate trichomes. Pubescence of this type was recorded as 1. The under side of the leaves of *Q. ilicifolia* is densely covered with a mass of whitish stellate trichomes, a type of pubescence recorded as 3. Intermediate types of pubescence were recorded as 2.

5. *Length of mature terminal buds of adult twigs.* The buds of *Q. marilandica* are usually about twice as long as those of *Q. ilicifolia,* and the two species are more sharply separated from each other in this than in any other measurement made.

6. *Depth of acorn cups.* The acorn cups of the two species are of about the same diameter, but those of *Q. marilandica* are deeply turbinate in shape, while those of *Q. ilicifolia* are usually flatter. The depth of the acorn cup, measured from rim to base, is therefore the measurement which differentiates the two species most strongly in this character.

In addition, the two species differ from each other in the number of and shape of the leaf lobes, the color of the bark, the pubescence on the bud and acorn cup scales, the shape of the cup scales, the character of the apex of the acorn, and a number of other characters less easy to define. In all of these it was so much more difficult to characterize each specimen than in the six characters selected, that they were not analyzed.

The distribution of classes of variants in respect to the four characters measured quantitatively is shown in tables 1 to 4. From these tables it is evident that in two characters, leaf width and bud length, the Lakehurst population contains a relatively high proportion of individuals with values intermediate between those characteristic of *Q. marilandica* and *Q. ilicifolia.* The same is true to a slight degree in depth of acorn cup, while in respect to number of veins in the lower part of the leaf the modal values for the two standard series are so close together than no intermediate categories can exist. In all of the characteristics measured, therefore, the Lakehurst population shows a relatively high proportion of values intermediate between those typical for the standard series of *Q. ilicifolia* and *Q. marilandica.* Although with the size of sample available, the statistical significance of the individual differences in frequency could not be established, their consistent trend indicates that they are not due to chance alone, and that larger samples would show statistically significant differences.

TABLE 1. *Frequency distribution of leaf width*

| Maximum width, cm. | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard, *ilicifolia* | 3 | 6 | 4 | 2 | 4 | 1 | | | | | | | | | | 20 |
| Standard, *marilandica* | | | | | 1 | 1 | 7 | 5 | 1 | 3 | 1 | 0 | 1 | | | 20 |
| Cliffwood | | | | 1 | 1 | 3 | 4 | 4 | 3 | 3 | 0 | 2 | 2 | | | 23 |
| Lakehurst | 4 | 9 | 8 | 8 | 12 | 11 | 14 | 12 | 1 | 2 | 2 | 0 | 2 | 0 | 1 | 86 |

TABLE 2. *Frequency distribution, number of veins on lower part of leaf*

| Number of veins | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Totals |
|---|---|---|---|---|---|---|---|---|
| Standard, *ilicifolia* | | | 6 | 14 | | | | 20 |
| Standard, *marilandica* | | | 1 | 1 | 10 | 7 | 1 | 20 |
| Cliffwood | | | | 2 | 10 | 9 | 2 | 23 |
| Lakehurst | 1 | 0 | 7 | 30 | 30 | 12 | 6 | 86 |

TABLE 3. *Frequency distribution, length of terminal bud*

| Length, mm. | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard, *ilicifolia* | 7 | 11 | 2 | | | | | | | | | 20 |
| Standard, *marilandica* | | | | | 1 | 7 | 4 | 5 | 2 | 0 | 1 | 20 |
| Cliffwood | | | | 2 | 1 | 3 | 8 | 4 | 2 | 2 | 1 | 23 |
| Lakehurst | 1 | 9 | 14 | 10 | 8 | 18 | 15 | 8 | 3 | | | 86 |

TABLE 4. *Frequency distribution, depth of acorn cup*

| Depth, mm. | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Standard, *ilicifolia* | | 1 | 6 | 5 | 2 | 3 | 1 | 0 | 2 | | | 20 |
| Standard, *marilandica* | | | | 2 | 2 | 6 | 1 | 2 | 5 | 0 | 1 | 19 |
| Cliffwood | | | 1 | 2 | 0 | 1 | 5 | 0 | 2 | 2 | 1 | 14 |
| Lakehurst | 1 | 6 | 7 | 11 | 10 | 21 | 17 | 7 | 5 | 1 | 0 | 86 |

In respect to one of the characteristics which was estimated qualitatively, namely character of leaf base, the distribution in the Lakehurst specimens is essentially the same as in the standard series. Standard *Q. ilicifolia* contained 10 specimens with base type 2, and 10 with type 3, while in *Q. marilandica* types 1 and 2 were represented by 10 specimens each. Thus in the two standard series added together the proportion of types 1:2:3 is 10:20:10. In the Lakehurst series the distribution of types 1, 2, and 3 was 29:44:13. The relatively high frequency of type 1 and the corresponding deficiency in type 3 is due to the fact that, as will be shown below (figure 3), individuals of *Q. ilicifolia* are relatively less abundant in the population than are those of *Q. marilandica*.

The other quantitative character, pubescence of the under side of the leaf, was the most significant of all, since in the standard series the intermediate type, 2, was not found at all. All of the *Q. ilicifolia* were type 3, while the *Q. marilandica* specimens were consistently type 1. On the other hand, 23 of the 86 specimens of the Lakehurst population, or 27 per cent, had type 2 pubescence on the under sides of their leaves.

The Cliffwood population showed essentially the same distribution in all of the characters measured as the standard series of *Q. marilandica*. The slight deviations in the direction of intermediacy seen in leaf width and terminal bud length are far from significant. On the other hand, 4 of the 23 specimens had pubescence of type 2, and therefore varied in the direction of *Q. ilicifolia* in this particular character.

The most logical conclusion on the basis of intermediacy in several otherwise unrelated characters is that some of the individuals in the Lakehurst population are either $F_1$ hybrids between *Q. ilicifolia* and *Q. marilandica*, or else segregating or backcross derivatives from such hybrids. This is particularly likely if individuals intermediate in one of the characters studied are intermediate also in one or more of the others. A rather crude and partly subjective but nevertheless satisfactory way of estimating this situation is provided by the aggregate hybrid index of Anderson (1936). This index was therefore computed for each individual. A series of standard scoring values was arbitrarily established, as shown in table 5. Comparison of this and the previous tables will show that low values for each character are typical of *Q. marilandica*, and high ones of *Q. ilicifolia*. The characters are not given equal weight. Since pubescence and bud size are most sharply distinct in the two standard series, the range of scores for

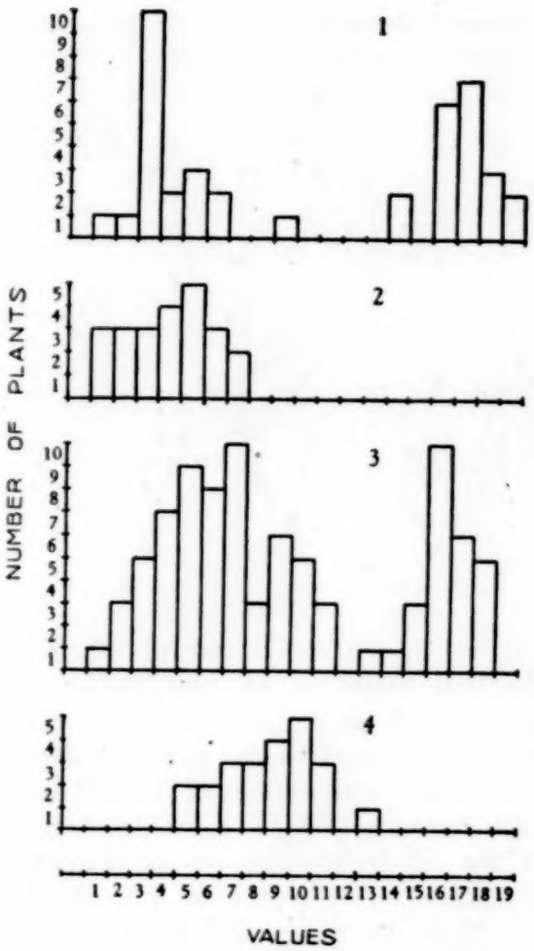


FIG. 1. Frequency distribution of index values of standard series of *Quercus marilandica* left, and *Q. ilicifolia* right.

FIG. 2. Frequency distribution of index values of Cliffwood population of *Q. marilandica*.

FIG. 3. Frequency distribution of index values of Lakehurst population of *Q. marilandica-Q. ilicifolia*.

FIG. 4. Frequency distribution of type 2 pubescence in Lakehurst population.

these two characters is respectively from 0 to 4 and 0 to 5. In leaf width, number of veins, and depth of acorn cup a moderate amount of overlapping exists between the two species, so that the range of scores for these characters is from 0 to 3. Finally, for the least sharply distinct of the six characters, type of leaf base, the scores assigned range only from 0 to 2.

The hybrid index values obtained for the standard, Cliffwood, and Lakehurst series are given in figures 1 to 3. The standard values, as expected, are markedly discontinuous, with 1 to 9 as index values for *Q. marilandica* and 14 to 19 for *Q. ilicifolia*. This shows that in spite of overlapping in individual characters, the two standard series of specimens are amply distinct in the aggregate of the six characters studied. In other words, specimens of the *Q. marilandica* series which approach *Q. ilicifolia* in leaf width or any other single character are essentially like their own species in most or all of the other characters. The Cliffwood sample has the same range of scores as the standard of *Q. marilandica*, but there is a slightly larger number of individuals with relatively high scores, that is, 5 or over.

The distribution of scores in the Lakehurst population, on the other hand, differs markedly from the standard series. It is still distinctly bimodal and discontinuous, since the score of 12 is not represented, but the series of index values from 9 to 13 is here represented by 15 individuals. Furthermore, the modal scores for *marilandica*-like individuals in this population are 5, 6, and 7, while that for the standard series of *Q. marilandica* is only 3. The hybrid indices, therefore, strongly suggest the presence of hybridization between *Q. ilicifolia* and *Q. marilandica* at the Lakehurst locality.

Further evidence for this hybridization was obtained by reexamining the particular individuals with intermediate scores. All specimens with index values of 10, 11, and 13 showed evidence of intermediacy in the lobing of the leaves, pubescence of twigs, of bud scales, and of cup scales, and shape of cup scales. Leaves of three individuals with these intermediate scores, along with those typical of *Q. marilandica* and *Q. ilicifolia*, are shown in figures 6, 7, and 8.

From the above data, it seems fairly clear that the 8 individuals in the Lakehurst population with hybrid indices of 10 or 11, which are intermediate between the scores of the two standard series, are either $F_1$ hybrids or progeny of such hybrids which have retained an approxi-

TABLE 5. *Index values for six character differences between* Quercus ilicifolia *and* Q. marilandica

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1. Leaf width | cm. | 13–18 | 10–12 | 7–9 | 4–6 | | |
| | score | 0 | 1 | 2 | 3 | | |
| 2. No of veins | no. | 6–7 | 5 | 4 | 3–1 | | |
| | score | 0 | 1 | 2 | 3 | | |
| 3. Leaf base | type | 1 | 2 | 3 | | | |
| | score | 0 | 1 | 2 | | | |
| 4. Pubescence | type | 1 | 2 | 3 | | | |
| | score | 0 | 2 | 4 | | | |
| 5. Bud size | mm. | 9–12 | 8 | 6–7 | 5 | 3–4 | 2 |
| | score | 0 | 1 | 2 | 3 | 4 | 5 |
| 6. Depth of cup | mm. | 13–16 | 11–12 | 9–10 | 6–8 | | |
| | score | 0 | 1 | 2 | 3 | | |

mately equal proportion of genes from the two parental species. Specimen no. 54, with an index value of 13, is probably a backcross derivative with predominantly genes of *Q. ilicifolia*. The 6 specimens with an index of 9 are very likely the result of segregation in the direction of or backcrossing with *Q. marilandica*, while among the 30 specimens with scores of 5, 6, 7, and 8 there may be other backcross derivatives. The number of these backcross types may be inferred in two ways. In the first place, it might be assumed that all of the individuals with type 2 pubescence on the under surface of their leaves, a type not found in the standard series, are hybrids or hybrid derivatives. As shown in figure 4, this type of pubescence is found in all but two of the individuals with scores 9, 10, and 11, and in 10 of those with scores of 5 to 8. It is possible, therefore, that these ten, plus all with the score of 9, or 16 in all, are backcross types. Among those with scores of 5 to 7, there are 7 with type 2 pubescence.

The second way of inferring the number of backcross types in the group with high *marilandica* scores is to estimate the deviation from the number expected in this group on the assumption that the typical distribution of scores in *Q. marilandica* is that represented by the standard series. Since 6 out of 20 of the standard series of *Q. marilandica* have scores of 5 or higher, we might expect that 30 per cent of the pure *marilandica* individuals with scores of 1 to 9 would have scores of 5, 6, 7, 8, or 9. Actually, of the 52 individuals in the Lakehurst population with scores of 1 to 9, there are 36 in the range of 5 to 9 instead of the expected 16. This indicates that a maximum of 20 of the Lakehurst individuals with scores of 5 to 9 may have some genes from *Q. ilicifolia*.

To summarize: in the Lakehurst population there are 8 individuals, or 9.3 per cent, which are essentially intermediate between *Q. ilicifolia* and *Q. marilandica*, and are almost certainly hybrids or hybrid derivatives. In addition one or two individuals, or a maximum of 2.3 per cent, are essentially *Q. ilicifolia* but with some genes from *Q. marilandica*, while between 16 and 20 individuals, or from 19 to 23 per cent, are probably *Q. marilandica* with some genes from *Q. ilicifolia*. Of the remaining 56 individuals, 24 are pure *Q. ilicifolia* and 32 pure *Q. marilandica*. The proportion of individuals with some genes from both species therefore lies somewhere between 30 and 35 per cent of the Lakehurst population. By far the majority of these are plants which are predominantly *Q. marilandica*

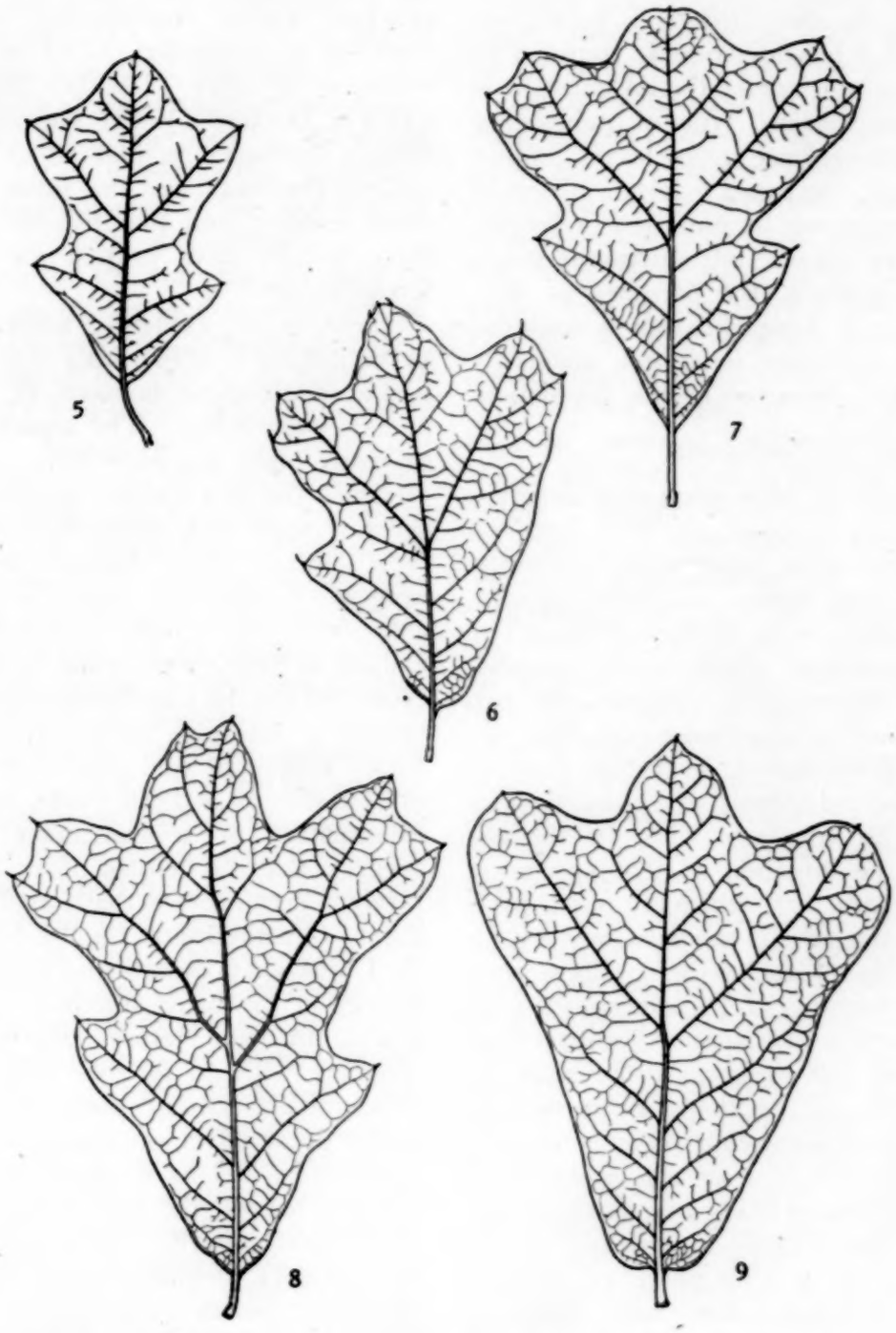


FIG. 5. Leaf of *Quercus ilicifolia*, from plant with index value 17 in Lakehurst population. $\times \frac{2}{3}$.

FIG. 6. Intermediate type of leaf from plant with index value 10 in Lakehurst population. $\times \frac{2}{3}$.

FIG. 7. Intermediate type of leaf from plant with index value 10 in Lakehurst population. $\times \frac{2}{3}$.

FIG. 8. Intermediate type of leaf from plant with index value 11 in Lakehurst population. $\times \frac{2}{3}$.

FIG. 9. Leaf of *Quercus marilandica*, from plant with index value 3 in Lakehurst population. $\times \frac{2}{3}$.

but which have a few genes from *Q. ilicifolia*. In the Cliffwood population evidence for hybridization is confined to the presence in four of the 23 individuals of the intermediate type of pubescence on the under surface of the leaves. Since this single character deviation could easily have arisen by mutation or recombination without the introduction of genes from *Q. ilicifolia*, the presence of *ilicifolia* genes in this population is doubtful, and they certainly have not materially affected the character of the population.

## Discussion

The evidence here presented indicates that where *Quercus ilicifolia* and *Q. marilandica* grow together hybridization between them occurs, and thus supports the observations of Davis (1892). The eight specimens which were morphologically intermediate between the two species could be classified as x*Q. Brittonii* Davis. Furthermore, a certain percentage of the individuals of *Q. marilandica* growing in company with *Q. ilicifolia* vary in the direction of that species in several characters, which in other parts of the range of *Q. marilandica* vary independently of each other. These *ilicifolia*-like variants of *Q. marilandica* form a more or less continuous transition from the exact intermediates to typical *Q. marilandica*. They therefore correspond in every way to the examples of introgressive hybridization described by Anderson and Hubricht (1938) in *Tradescantia*, and by Riley (1938) in *Iris* and (1939) in *Tradescantia*. To prove conclusively that these intermediate individuals are hybrids and hybrid derivatives, the cross would have to be repeated artificially. But in the case of other suspected hybrids between oak species, progeny tests have demonstrated the presence of segregation for interspecific differences (MacDougal, 1907; Allard, 1932; Yarnell, 1933; Stebbins unpubl.) so that genetic evidence has established the existence of occasional natural hybrids between species of *Quercus* beyond reasonable doubt, and has shown that such hybrids can produce viable, segregating offspring from open pollination. The cytological evidence of Sax (1930), Natividade (1937), Duffield (1940) and others indicates, furthermore, that all of the species of *Quercus* have the same chromosome number, and that hybrids between them usually have normal meiosis and fertile pollen.

There is no evidence, however, that hybridization is obliterating the morphological distinctness between *Q. ilicifolia* and *Q. marilandica*. The writers as well as many other botanists have commonly observed the two species occurring intermingled with each other throughout the New Jersey pine barren area, and everywhere they are easily distinguishable from each other. Whether the amount of hybridization and backcrossing estimated for the Lakehurst population is typical for the pine barren area of New Jersey cannot be told until more population studies have been made, but casual examination of several other localities suggests that the number of intermediates rarely if ever exceeds that found at Lakehurst. Furthermore the evidence from the Cliffwood population as well as casual inspection of many stands of *Q. marilandica* in New Jersey growing not in company with *Q. ilicifolia* indicates that genes of the latter species have had a purely local effect on populations of *Q. marilandica* and have spread little if any distance beyond the region of occurrence of *Q. ilicifolia* itself. The amount of gene flow from *Q. ilicifolia* into *Q. marilandica* thus corresponds rather closely with that recorded by Epling (1947) for genes of *Salvia apiana* into *S. mellifera* in California.

No evidence has been obtained for the flow of *marilandica* genes into *Q. ilicifolia*. The absence of this in the Lakehurst population is most likely due to the fact that most of the $F_1$ hybrids occurred in a part of the colony in which *Q. marilandica* was much more abundant than *Q. ilicifolia*, and therefore backcrossing

with the former species would have a far greater probability. In the region where *Q. ilicifolia* was most abundant, it was growing in a dense stand somewhat isolated from the main body of *Q. marilandica*. It is possible that in other colonies introgression from *marilandica* into *ilicifolia* can be demonstrated.

Although the present example involves only one population and two species of the genus *Quercus*, the situation described here is more or less typical of that found in the genus as a whole, at least in North America. Almost everywhere two or more related species of oaks occur sympatrically, and occasionally hybridize. Rarely, however, does this hybridization affect the essential integrity of the species, even though the hybrids are fertile and a certain amount of segregation and backcrossing occurs. In addition to geographical isolation and hybrid sterility there must be other isolating mechanisms at work to maintain the distinctness of the various species of *Quercus*. Seasonal isolation is of little or no importance, since related species usually shed their pollen at about the same time in the spring, and in these wind-pollinated species mechanical isolation is non-existent, so that the stigmas of any oak species in its natural habitat must receive pollen from foreign species with a very high frequency.

Three isolating barriers remain to be considered, namely ecological isolation, incompatibility of foreign pollen, and reduced viability of hybrid embryos. Although no direct evidence is available, indirect evidence suggests that all three of these may be operating. The number of $F_1$ hybrids in the Lakehurst colony must be small, since some of the 8 intermediate individuals are almost certainly $F_2$ and later generation segregates. This suggests that either interspecific cross pollination leads to fertilization considerably less often than cross pollination between members of the same species, or that the majority of hybrid embryos and seedlings are less viable than embryos or seedlings of the parental species. Nevertheless, the number of intermediates is far larger than in most other populations of sympatric species, particularly in animals, and is large enough so that one might expect complete obliteration of the species barrier in relatively few generations if seedlings derived from segregation and backcrossing had a selective value equal to that of the pure species. The number of generations during which the colonies of *Q. ilicifolia* and *Q. marilandica* have existed in New Jersey is hard to estimate. Although the aerial trunks are destroyed by fire every ten to twenty years and sometimes more often, the stumps of both species survive this treatment, and seedlings are uncommon, even after fires or other destruction of the trees. It is possible, therefore, that the present individuals have existed as roots and stumps for hundreds of years. Even in the 25,000 years since the close of the Pleistocene the number of generations might have been no more than 250, and for various reasons perhaps not nearly that many. A conservative estimate of the maximum number of generations during which the Lakehurst and other populations have existed might therefore place this number at 250, and it may well be considerably less than a hundred. In other words, given an equal rate of change per generation, the total amount of alteration of gene frequencies which could have been accomplished in this population during 25,000 years or less is about the same as that which could occur in *Drosophila* in about ten years.

When these facts are considered, it is clear that even a slight discrimination against seedlings of hybrid derivation, due to the unavailability of suitable ecological niches for them, could check the rate of gene flow between the two species populations to a sufficient degree so that merging would be exceedingly slow. And the fact that *Q. marilandica* and *Q. ilicifolia* have in general different geographical ranges, and even in New Jersey

occupy somewhat different habitats, suggests that such ecological selection pressure against hybrid types may exist.

The present study is reported not as a finished piece of work which claims to give a definite answer to any of these problems, but it is intended to suggest ways of exploring further the nature of species and of species barriers in this most important and interesting genus of flowering plants.

## Summary

Population samples of *Quercus marilandica* and of *Q. ilicifolia*, gathered at Lakehurst, N. J., and of *Q. marilandica* from Cliffwood, N. J., were compared with herbarium sheets of these two species collected for the most part outside of the region of their geographical and ecological overlap. The following characters especially were studied: leaf width, number of veins on lower part of leaf, character of leaf base, pubescence of under surface of leaves, length of mature terminal buds of adult twigs, and depth of acorn cups. Hybrid indices were computed; the Cliffwood material had the same general range of scores as the standard of *Q. marilandica;* in the Lakehurst population approximately 37 per cent of the individuals collected were *Q. marilandica,* approximately 28 per cent were *Q. ilicifolia,* and the remainder showed varying degrees of intermediacy, the gene flow in this particular sampling being predominantly from *Q. ilicifolia* into *Q. marilandica.*

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# EXPERIMENTAL STUDIES ON ADAPTIVE EVOLUTION IN *GASTEROSTEUS ACULEATUS* L.

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## INTRODUCTION

The complex of populations referred to as the Three-spined Stickleback, *Gasterosteus aculeatus*, presents an exceptional challenge to the student of evolution. Two modes of morphological variability exist in this complex, each correlated with definite ecological characteristics, and each occupying areas where the other one is rare or absent. These two morphological and ecological modes are, however, connected by a series of intermediate forms that are found in a boundary zone in which the distributions of the two principal groups of populations come in contact. The puzzling aspect of the situation is that the morphological intermediates are not necessarily intermediate in their physiological properties and in their ecological requirements.

The purpose of this paper is to report observational and experimental evidence that appears to throw light on the origin of the differentiation among the populations of *Gasterosteus aculeatus*. This differentiation seems to be caused by natural selection which favors different complexes of genes in the different ecological niches in which the species occurs.

## I. DISTRIBUTION OF PLATE NUMBERS IN NATURAL ENVIRONMENTS OF DIFFERENT TEMPERATURES AND SALINITIES

A biometrical analysis of the stickleback populations throughout Europe was made by Bertin (1925) who concluded that there exists a simple correlation between the number of plates on the body of the fish (figure 1) and the environment in which it lives. He believes that the plate number decreases regularly and continuously from north to south and from waters with high salinities to those with low ones. These correlations and gradients are interpreted by Bertin in light of his Lamarckian concepts.

Extensive sampling and biometrical study of the stickleback populations in Belgium (Heuts, 1947) showed that Bertin's interpretations suffer from considerable oversimplification. The stickleback populations of Europe do not present a picture of continuous variability in either morphological traits or in ecological requirements. Instead, we find two types of populations that form sharply bimodal curves. One type of populations

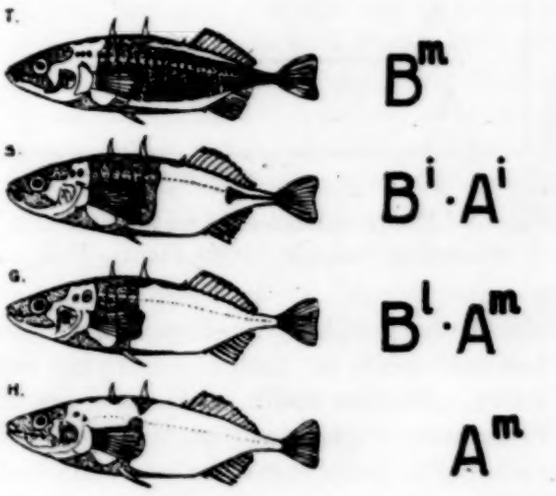


FIG. 1. The four types in plate number variants as conceived by Bertin. The corresponding nomenclature adopted in this paper is indicated. The $A^m$ type without plates is found only at the southern distribution limit of the species.

[1] Bevoegdverklaard Navorscher bij het Belgisch Nationaal Fonds voor Wetenschappelijk Onderzoek.

consists of fishes with a modal number of about 32 plates and the other type is characterized by a mode of about 5 plates (figure 2). Individuals intermediate between these modes do occur, but they are not frequent in any populations. Populations consisting entirely of individuals with a high number of plates occur chiefly in extreme northern Europe. From southern Scandinavia southward the stickleback populations contain more and more intermediates, and populations with a low number of plates also appear. In Belgium populations with a high number of plates occur mainly in the vicinity of the sea, in waters of considerable to high salinity. Populations with low numbers of plates occur in fresh water. Farther south, in France, populations with high mean plate number increasingly disappear from brackish water and become more and more restricted to marine habitats.

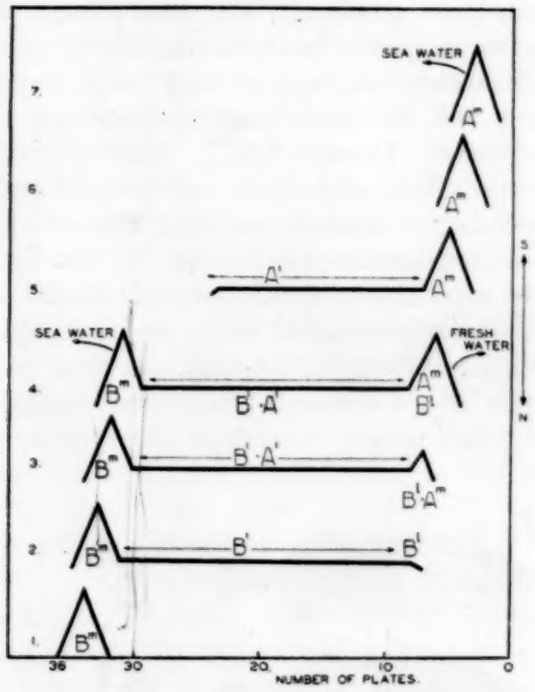


FIG. 2. The frequencies of plate numbers in relation to latitude (from Heuts, 1947).

The fresh water populations of Belgium, and probably also of Holland and northern Germany, are characterized by low numbers of plates, with modes from 5 to 7 in Belgium. In some of these populations intermediates are also found but they occur only with low frequency. The body size of fishes of these populations is smaller, the approximate ratio to the mean size of the northern populations being as 3:4. From mid-France these populations with low plate numbers entirely replace the northern ones.

For convenience of reference I have adopted the following nomenclature (figure 1). The fishes of populations with low means for plate number and with small body size I have designated as A types, those from populations with high means for plate number as B types. Within the A types, I have distinguished those that are distributed around the mode as $A^m$, those with higher plate numbers and a caudal crest as $A^l$. Similarly for the B types, those around the mode are $B^m$, the intermediates $B^l$ (with a caudal crest) and the $B^l$ types, that are identical with the $A^m$ types in plate number, but not in size.

Apart from the differences in size, the two types differ also in other characters (Bertin, 1925). In Belgium there are mean differences in vertebral numbers (Heuts, 1947). Differences in breeding habits also exist in Belgium, where the B type is known to perform annually in autumn katadromous migrations, to reach maturity in the sea, and to return to the breeding grounds to spawn during the following spring. Type A, on the contrary, always remains in fresh water.

Figure 2 represents the variability in plate numbers of the whole species with respect to latitude. From this it will be seen that the $B^m$ types as well as the $A^m$ show a cline from north to south within their respective territories. The mean plate number in type $B^m$ ranges from about 34 to 31, that of type $A^m$ from 7 to 3 (or to zero farther south, figure 1). As these changes in morphology occur from north to south, each type becomes increasingly restricted to brackish and salt water. Type A is found in salt water in southern France. The Belgian popula-

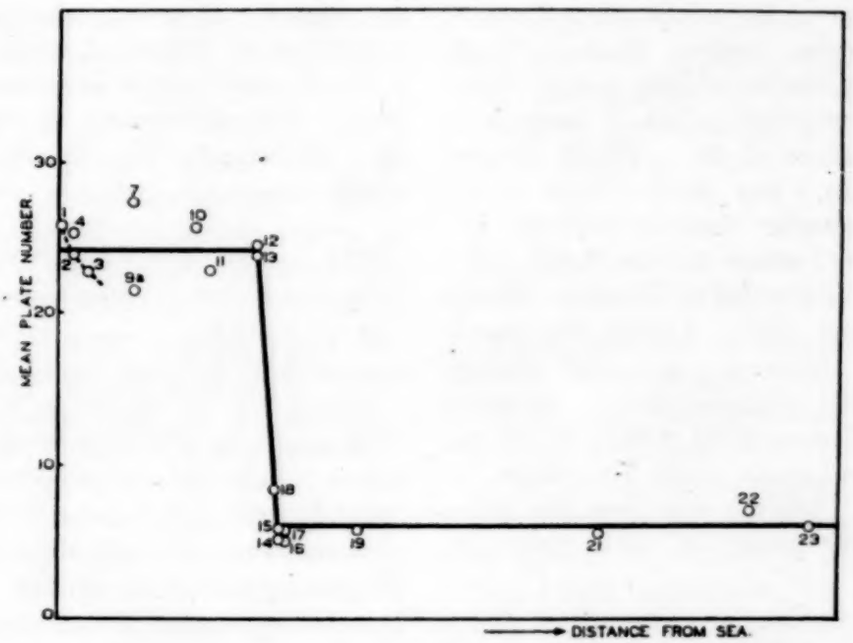


FIG. 3. The variation of the means of plate numbers of 23 populations found in Belgium, proceeding from the sea shore inland (from Heuts, 1947).

tions, which are situated centrally, comprise both types (graph 4, figure 2) in approximately equal numbers.

The two types also differ in Belgium in their seasonal distributions. In winter, type B is wholly restricted to the sea, and in spring and summer to the rivers of the western part of Belgium at short distances from the sea. Type A is confined to the eastern part of Belgium at all times, and lives in small creeks the salinity of which may be assumed to be very low. Within a given drainage, the two types may approach each other but scarcely mix. This is shown in figure 3, where the mean plate numbers of a series of population samples numbered from 1 to 23 are charted according to locality from western to eastern Belgium. From these, and other data presented by Bertin, it is clear that the plate number is correlated in nature with salinity and with temperature of the habitat. However, as shown by figure 3, no regular cline exists at a given latitude with respect to the salinity. The sharp break in the gradient shows that no intermediate populations occur. The same is true of temperature gradients at a given salinity. Observational evidence for the constancy of plate number within the individual lifetime is given in Heuts (1944b); experimental evidence will be published elsewhere.

## II. Correlation of Plate Number with Salinity and Temperature as Determined Experimentally

The fact that the various morphologically characterized populations of sticklebacks are confined to different habitats, as shown in the preceding section, indicates that these populations differ in physiological characteristics that are correlated with the morphological ones. The distribution of the morphological variants being a function of temperature and salinity, it is only logical to seek the cause in the processes that regulate the ion and water exchanges of the organism, that is, their osmoregulatory processes.

In a study of chlorine regulation of the blood at different temperatures (Heuts, 1945) I have shown the existence of great differences in the regulatory capacities of individuals from two populations in Belgium (types A and B). The most conspicuous fact detected was the com-

plete inability of B individuals to maintain the chlorine content of their blood when kept in waters of low salinity (external concentration below ¼ seawater) at a temperature of 20°. Death always follows within a few days. Type A individuals, however, have a chlorine retaining power which at the same temperature can withstand fresh water diluted with redistilled water. During the breeding season, however, a cyclic change occurs in the osmoregulatory function (Koch and Heuts, 1942, 1943) which results in the anadromous migration of these fishes. During this time the chlorine retaining power of both types increases.

It was possible to show that these physiological differences are correlated with plate number, first, in relation to salinity, and second, in relation to temperature. Samples of wild populations ($N = \pm 200$) from different localities in Belgium were gathered in winter, when they consist only of adult but sexually immature animals. Only populations of this kind can be used because individual differences in sexual maturity, which would exist in spring, modify the physiological reactions that were investigated. In summer, differences in age would obscure the results even more.

Each collected sample was brought to the laboratory and immediately subjected to extreme environmental conditions, differing in each of the described experiments. As a result, the fishes died differentially over a period of 36 hours or more. Each hour the dead fishes were removed and their characters submitted to biometrical analysis. The results of three typical experiments follow. The characters of the populations employed are discussed in detail in Heuts, 1947, as indicated.

A sample of 192 type $A^m$ individuals (population Nr. 17, Heuts, 1947) was transferred suddenly from fresh water to seawater (35 $^0/_{00}$ salinity) at 10° C. The relation of plate number to survival under these conditions is shown in table 1. The experiment was continued for 48 hours, at which time some of the animals of the last class were still alive. The differences in survival time are statistically significant, P being smaller than .01.

TABLE 1. *Survival of $A^m$ in seawater at 10° C.*

| Number of plates | Mean hours of survival |
|---|---|
| 3 and 4 | 7½ |
| 5 and 6 | 11 |
| 7 and more | 13¾ |

A sample of 175 type $A^m$ fishes (population Nr. 21, Heuts, 1947), collected in January in water of about 0° temperature, was subjected to fresh water at 25°–28°. Deaths occurred as shown in table 2. In this experiment P is also less than .01.

TABLE 2. *Survival of $A^m$ in freshwater at 25°–28° C.*

| Number of plates | Mean hours of survival |
|---|---|
| 3 and 4 | 43 |
| 5 | 39 |
| 6 | 38½ |
| 7 | 36¾ |

These experiments show that, in high salinities at low temperature, the fishes with higher plate numbers are favored, while those with lower plate numbers are selected against. Plate numbers may therefore be taken as an index of the selective value of the correlated physiological characters. The same can be said for high temperature in fresh water, as shown by the second experiment. There, however, the lower plate numbers have the selective advantage.

This correlation occurs only within the $A^m$ type, that is in those individuals distributed around the mode, but not in the high extremes, which approach the plate number of the $B^m$ type. When a population which consisted of approximately equal numbers of $A^m$ and $A^l$ types (that is, including these extremes) was subjected to the same conditions as in experiment 1, that is to say 35 $^0/_{00}$ salinity at 10° C., no association was found between plate number and survival time. This is apparently due to the fact

that the maximum resistance to increased salinity is reached at the point where the plate number is highest for $A^m$, and is maintained despite a further increase in plate numbers in $A^l$ types. This is to say that the maximum selective value of the physiological characters seems to be reached at a point below the maximum value of the morphological characters, or at least that after a given point the increase of the selective value is no longer a linear function of the plate numbers.

It would be highly desirable to ascertain the differences in reactions of the B populations in a similar fashion, but this is impossible for the reason that sufficiently large population samples of adult but sexually immature fishes are not available in winter, because of their restriction to the ocean at that time. No inference is possible, therefore, as to similar selective differences within $B^m$ types. From data to be discussed presently (egg survival), the conclusion is justified that the selective values of B and A intermediates differ widely despite their similarities in plate numbers.

Because of restriction to the ocean in winter, it is also impossible to compare directly the A and B types by this method of differential survival. However, from the results of other methods described in Heuts (1945), it becomes clear that the physiological differences between the A and B types are so sharp that the results of such a selection experiment can be foreseen. They indicate that, if A and B types were transferred together to fresh water at a temperature as low as 20° C., all B types would be killed, while A types would survive. Similarly, a sudden transfer to seawater would not affect the B types, while A fishes would die. Despite the fact that all their morphological characters including plate numbers intergrade, the physiological differences between A and B types are much greater than the differences existing within the two types. All these facts are in complete accord with the data on the geographical distribution of the two types. The question then arises whether the plate numbers of these populations are simply induced by the differences in environment, or inherited and subject to natural selection.

## III. Inheritance of Plate Numbers and Salinity—Temperature Tolerance

Large numbers of zygotes of known parents were obtained through artificial fertilization of the stickleback by methods described by Leiner (1934). Eggs from parental pairs, the numerical and quantitative characters of which were recorded, were placed separately in small baskets of fine mesh, black enameled, wire screen. These baskets were suspended in aquaria, so that the eggs were near the surface. The aquaria were placed in constant temperature rooms and the water was continuously aerated. Seawater was prepared artificially (formula of Schmalz). Redistilled water was used to make all dilutions of seawater and of fresh water. Fractions, as given throughout this paper, are such made with distilled water. The number of eggs hatching is high under these conditions of favorable combinations of temperature and salinity.

The larvae were left for two days in the baskets after hatching. Then they were counted and killed, or transferred gradually (with respect to temperature, salinity and hydraulic pressure) to large aquaria. The aquaria previously had been kept in full sunlight, so that a rich microfauna and flora had developed. This condition is necesary for successful rearing of the offspring. The larvae were fed on powdered eggs.

Parents from the same two populations obtained near Louvain were used in the following experiments. One population belonged to the large-sized group, comprising $B^m$, $B^l$ and $B^l$ fishes, the other comprised only $A^m$ fishes, as determined by samples made in successive years. The frequencies of the plate numbers in each of the two parental populations are shown in figure 4. At each combination

of temperature and salinity, several pairs of parents of each population were used. The total number of eggs on which the following results are based was approximately 75,000.

Each female produces 100 to 400 eggs at each spawning. The number of eggs produced by B females is always considerably higher and generally double the number produced by A females. In the earlier experiments we were not sure enough offspring that would reach adult size could be obtained. Therefore the offspring of three matings of the same kind were put in one aquarium, in which case the initial population of one aquarium may have been as many as 600 indi-

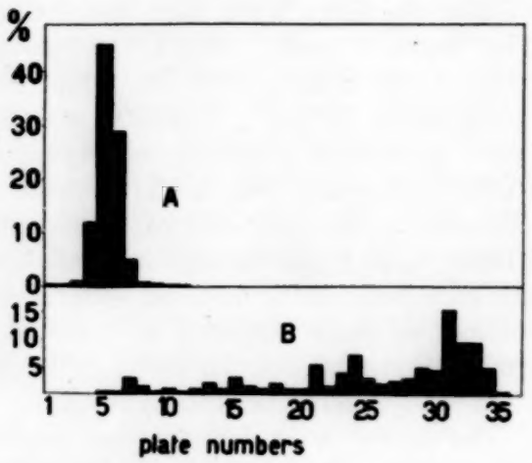


FIG. 4. The frequencies of plate numbers in the two parental populations.

viduals. Because of the crowding, only a small fraction became adults. Later, the offspring of only one parental pair was put in one aquarium and the percentage of larvae reaching adult stage was greatly increased.

The first series of experiments was carried out at 23° C., but the salinity varied from $\frac{1}{8}$ tap water with $\frac{7}{8}$ distilled water at one extreme, to seawater (33.5 $^0/_{00}$). The percentages of hatched eggs of both types are plotted against salinity in figure 5. The results show that at this temperature, type B eggs are tolerant of high salt concentrations, while type A eggs withstand only low concentrations.

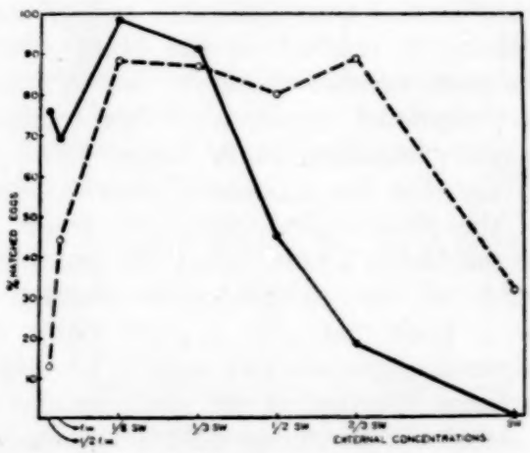

FIG. 5. Percentages of egg hatching at 23° in different salinities. ——●—— type A, —— ○ —— type B.

Whether such a high temperature occurs at the spawning grounds is doubtful. Considered from this standpoint, the results obtained at 19° are more significant. Figure 6 shows that at 19° the percentages of hatchings do not follow even gradients as is the case at 23°. Instead, sharply bimodal curves are observed for both types. Type A shows maxima of hatching at about 0.33 $^0/_{00}$ and at 10 $^0/_{00}$ salt concentrations, and a minimum at 3.3 $^0/_{00}$. The maxima of hatchings for type B are at about 0.04 and 3.3 $^0/_{00}$. In other words, the maxima

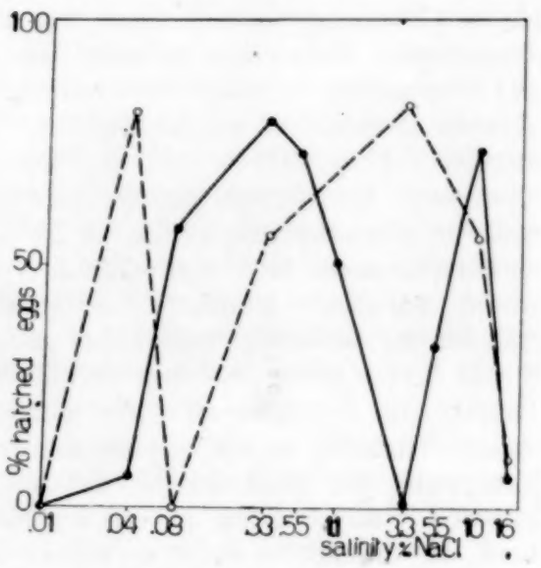


FIG. 6. Percentages of egg hatching at 19° in different salinities (on logarithmic scale). ——●—— type A, —— ○ —— type B.

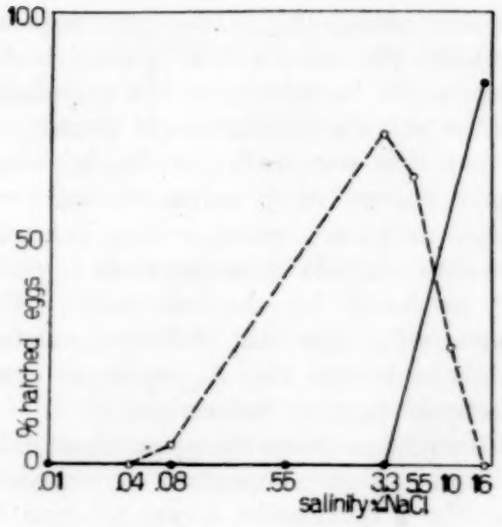


FIG. 7. Percentages of egg hatching at 10° in different salinities (on logarithmic scale). —●— type A, — — ○ — — type B.

for type A correspond to the minima for type B, and vice versa. This permits the two populations to occupy the whole range of habitats from fresh water to ½ seawater. They are, however, adapted to quite different concentrations within this range. At a still lower temperature, namely at 10°, a lower salinity is more favorable for hatching in type B and a higher one for type A (figure 7). In other words, the preferences at 10° are the reverse of those at 23° (figure 5), at which temperature, as shown above, type B prefers a higher salinity. At some intermediate temperature, the preferences must become reversed. But they must do this in such a way that the integrity of the adaptive systems is maintained. This is apparently accomplished by the selective preference to different concentrations, shown in figure 6. The data for completing the curve in figure 7 are not all at hand, but enough are present to justify the extrapolations. Figure 8 suggests the probable relationships of the adaptive peaks for temperature and salinity. This is based on extrapolation from the data at known temperatures. At 15°, some data were obtained for the A type, showing that for this type the picture is about the same at 19°. The adaptive peak at the lowest concentrations is, however, apparently breaking down, while the one at higher concentrations becomes steeper.

The inherited physiological differences that characterize the conditions of hatching are thus shown to form a complicated system. The tolerance ranges of the eggs are so narrow that natural selection can operate effectively under normal conditions. It is clear, however, that the selective value of either type A or type B cannot be defined in terms of the whole ranges, but only in terms relative to each combination of external conditions. Thus far, only differences in inherited physiological characters between A and B types have been considered. Other experiments show that selection in relation to plate numbers may also occur within population A although the intensity of this selection is relatively small. The analysis of the $F_1$ offspring of $A^m \times A^m$ crosses at 23° reveals interesting differences. The larvae hatching at different salinities, namely in fresh water, in $\frac{1}{6}$ seawater and ½ seawater (figure 5) were transferred into fresh water aquaria at room temperature. After one year their

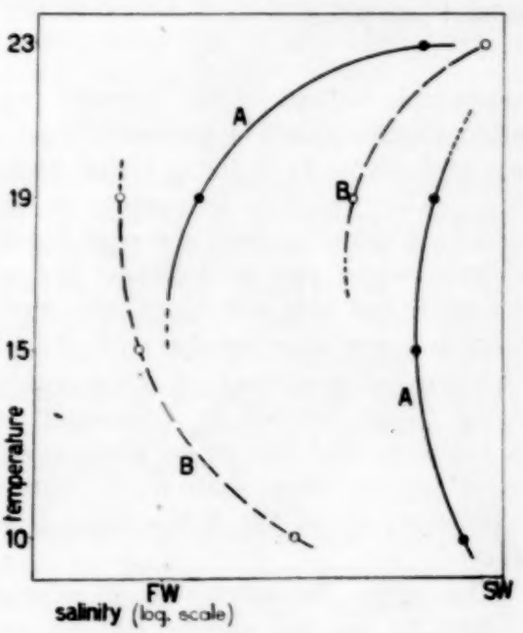


FIG. 8. The probable location of the adaptive peaks with respect to temperature and salinity. ——— type A, — — — — type B.

characters were analyzed. The frequencies of plate numbers in the adults which came from the eggs hatching in $\frac{1}{6}$ seawater (figure 5) showed a variability

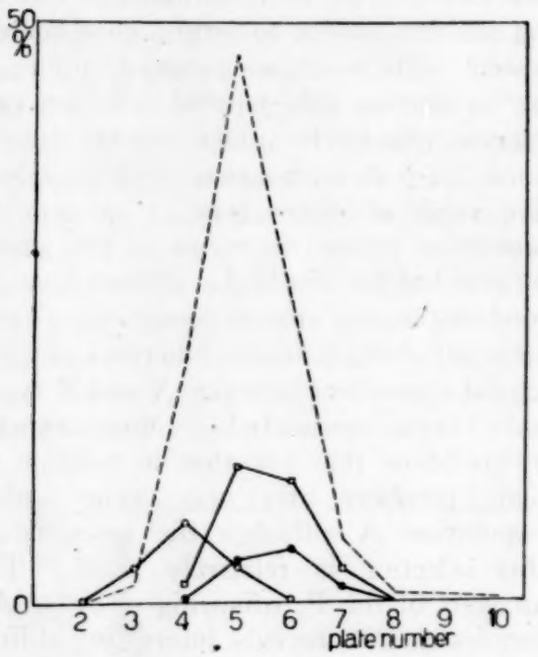


FIG. 9. Per cent survival of individuals with different plate numbers in the $F_1$-offspring of type A hatched in fresh water (——○——), in $\frac{1}{6}$ sea water (——□——), and in $\frac{1}{2}$ seawater (——●——). The dash curve shows the frequencies of different plate numbers in the parental population.

comparable to that of the parental population (figure 9). The greatest frequency was at 5 plates as in the parental population. The greatest frequency in individuals developing from the eggs hatched in fresh water was at 4 plates; not only the mode but also the range was shifted towards lower plate numbers ($P < 0.01$). The greatest frequency of plate numbers in the fishes, hatched in $\frac{1}{2}$ seawater, was at 6 plates, and the mean plate number has a higher value, than in the parental population, or in the fishes from $\frac{1}{6}$ seawater.

The eggs deposited in $\frac{1}{6}$ seawater (figure 5) hatched nearly 100 per cent, those deposited in $\frac{1}{2}$ seawater about 45 per cent, and those in fresh water 69 per cent. It can be assumed that the potential variability of the eggs with respect to the numbers of plates is the same as the variability in the population from which the parents were drawn. It follows that the deaths in the egg stage that occurred in $\frac{1}{2}$ seawater and fresh water were not random but selective. The observed shifts in numbers of plates are produced by this selection. This shows once more that different numbers of plates within the A population have different selective values.

Other facts from the same experiment indicate other important conclusions. Namely, a correlation seems to exist between the mean number of plates of each parental pair and the number of eggs hatched from them in a given environment. This is indicated in table 3 for the data obtained in fresh water and in $\frac{1}{2}$ fresh water, $\frac{1}{2}$ redistilled water (figure 5. The trend of the selective effects may be assumed to be the same at these two concentrations.

The correlation coefficient obtained is $-0.42$. However, since the total number of observations is small (15), the P value obtained is only slightly above .10.

TABLE 3. *Hatching in 1/1 and 1/2 freshwater*

| Mean parental plate number | Percentages hatching | | | | |
|---|---|---|---|---|---|
| | 41–52 | 53–64 | 65–76 | 77–88 | 89–100 |
| 4.5–4.9 | | | 1 | | |
| 5.0–5.9 | | 2 | | | 3 |
| 5.5–5.9 | | 2 | | 1 | |
| 6.0–6.4 | 1 | 2 | | | |
| 6.5–7.0 | | 2 | 1 | | |

The data obtained indicate that the population concerned was heterogeneous in its physiological characters as reflected in the different numbers of plates. It has been shown above that large physiological differences exist between the adults of types A and B. Within the type A smaller differences exist that show a strict correlation with plate numbers. These results were obtained only

by exposing the adults to extreme conditions, which rarely if ever are found in nature. In the conditions of hatching, I have found the same differences related to plate number in the same way, but at this stage of the life cycle the differences become apparent under combinations of salinity and temperature that are no more extreme than those found in nature. It thus becomes clear that the early stages of the life cycle provide a field of action for the operation of natural selection, and thus produce and maintain the divergence of the two types.

## IV. The Reduction of Gene Flow

### (1) *Selection*

The data considered above indicate that selection operating on the physiological characters of the eggs has produced two adaptive peaks, each characterized by a difference in mean plate number. But in respect to morphological characters at hand, a marked overlap is found. This might be interpreted as a result of hybridization, particularly since hybridization is possible after the spawning migration, at which time breeding occurs in the same regions (at least for some populations). It is, therefore, of interest to examine the reactions of experimental hybrids to different combinations of salinity and temperature. If these hybrids represent an adaptive type intermediate between the parents, then a continuous channel for gene flow would exist.

Reciprocal crosses between type A and B at 23° C. in salinities in which the selective values of the homozygous eggs differ widely gave the results shown in table 4.

These results show clearly a maternal influence (see figure 5). The selective values of the hybrid eggs are therefore not intermediate between the parents but are identical with those of the mother. Because of this mechanism, the building up of an intermediate adaptive peak is prevented.

TABLE 4. *Hatching of reciprocal crosses and of controls*

| Crosses | Mean hatching percentages | |
|---|---|---|
| | in fresh water | in ½ seawater |
| B♂ × B♀ | 44.0 | 80.0 |
| A♂ × B♀ | 46.3 | 80.5 |
| B♂ × A♀ | 91.3 | 45.8 |
| A♂ × A♀ | 69.0 | 45.3 |

Still another possibility for considerable gene flow may exist however. Although it may be inferred that the $B^1$ individuals have approximately the same physiological properties as the $B^m$ fishes, it is conceivable that they might produce eggs with intermediate selective values. However, in the experiment designed to ascertain the adaptive peaks of the B population, $B^m$ as well as $B^1$ and $B^1$ animals were used. Nevertheless, the sharp differences in selective values between A and B eggs were found. This proves that the eggs of $B^1$ fishes were not intermediate in physiological characters between A and B, regardless of the number of plates in the parents. It seems improbable therefore that the intermediates in nature would be gene carriers between the two populations to any larger extent than the extremes.

### (2) *Isolating mechanisms*

The selective mechanisms discussed above would seem to be sufficient to minimize but not to prevent gene flow between A and B populations for the following reasons. During the sexual maturation process a cyclic change in the osmoregulatory function takes place which results in the anadromous migration. This occurs chiefly in the B type (Koch and Heuts, 1942, 1943). At this time the differences in chlorine retaining power between the two types are notably smaller (Heuts, 1945). Thus although the breeding grounds are generally separate, during this critical period occasional individuals of type B may be found at the spawning places of type A and vice versa.

Therefore opportunity is present for a certain amount of cross breeding. Because the requirements of hybrid eggs are the same as these of the population from which the mother of these eggs came, there would probably be a loss of 50 per cent by reason of deposition in sites outside the adaptive peak of the females. Any mechanism that would prevent this loss of offspring would presumably have selective value (Dobzhansky, 1940).

Although it is true that occasional individuals are found in the wrong spawning areas, their number is far smaller, in my opinion, than would be expected in view of the relatively slight differences in chlorine retaining power at this season. Some other mechanism therefore must be operating which isolates the adults more effectively at that season and prevents a great wastage of eggs. This presumed mechanism must operate so as to distribute the adults in a way to coincide with the adaptive peaks of their respective eggs. It is conceivable that small differences in the concentrations of some ions may determine the segregation of the adults. I have shown, for example, that low Ca-concentrations influence the osmoregulatory mechanism of the stickleback (Heuts, 1944a).

The fact that individuals of both types may occur together does not itself insure interbreeding. Unfortunately, experimental data concerning sexual isolation are as yet too incomplete to permit any conclusion. Differences in breeding seasons would also provide an isolating mechanism. No marked differences exist however, although available data indicate that the stage of maturation of the eggs is correlated with plate numbers.

### (3) *The limitation of recombination*

One of the problems unsolved by the experiments discussed above is why intermediate types ($A^1$ and $B^1$) occur in nature, and since they do occur, why only at such low frequency. This low frequency might be explained by assuming that they are hybrids and, because of the mechanisms discussed, are seldom produced. But this assumption still does not explain why there are two physiological kinds of these intermediates, which are always or nearly always found with their respective modal types, with which they constitute, respectively, fully interbreeding populations. Nor does it explain why in each type the intermediates remain always at a constantly low frequency compared with the modal type.

There is no evidence that the $B^1$ types are at such a decisive selective disadvantage as to explain their rarity. Hence, the explanation must be in the existence of some particular genetic mechanism involved in the transmission of the plate number and physiological characters. Only two generations of crosses have as yet been reared, but I believe that the data are sufficiently informative in conjunction with other observational data.

The various crosses to be discussed were made in combinations of temperature and salinity which fell mostly within the adaptive peaks of the parents used. Selection is not likely therefore to have played an important part in the results. The results of a typical series are shown in figure 10. The cross *a* (in this chart) shows that $B^m♂ \times B^m♀$ produces $B^1$ as well as $B^m$ individuals. It must be borne in mind, therefore, that the $B^m$ parents are heterozygous. The desideratum would have been a homozygous line but this I have not yet obtained. The four following crosses (*b*, *c*, *d*, and *e*) were made with B type animals of different plate numbers, as indicated by the position of the parents in the chart. From these data it will be observed that the progenies have all the characters of remote outcrosses and that, instead of being intermediate, the mode tends to fall in the modes of the parental types. This is especially true when the cross was made with individuals of extreme B types (*e*). The same result was obtained when crosses were made between A ♂♂ and $B^1$ and $B^m$ ♀♀ (*f*). The parental types were reconstructed. The reciprocal cross

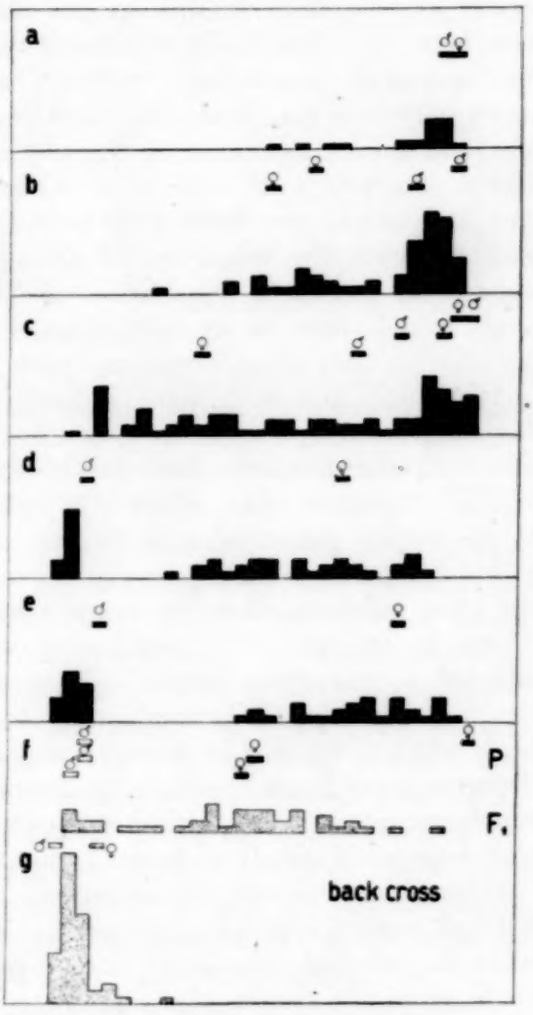


FIG. 10. Seven typical crosses (a to g) between carriers of different plate numbers. In black: B type. In white: A type. Dotted: $F_1$ or $F_2$ offspring from A × B crosses. The plate numbers of the parents are indicated by their respective positions. (The parental populations are shown in figure 4.)

is similar; hence there is no maternal effect, as observed in the early physiology of the egg (table 4).

From these crosses it seems likely that free recombination of the genes involved does not take place. The existence of two polygenic linkage groups, one containing largely + genes, the other − genes, for their respective types, is strongly suggested.

This explanation is sufficient to justify the assumption made previously, that the intermediates are in fact either $F_1$ hybrids, or $F_2$, or backcrosses. The intermediates represent chiefly crossovers between the two linkage groups. This may account for the low frequencies of $B^1$ types within the B populations and for the maintenance of the peculiar skewed distribution of plate number frequencies.

In figure 11 is presented the frequency distribution of the number of plates of all known B populations of Europe (Bertin, Boulenger, Heuts) in which $B^1$ and $B^1$ individuals are found. Occasional A types are also included. It will be observed that the frequencies of the $B^1$ and $B^1$ individuals fluctuate regularly in series of approximately 4 plates. This fact can be explained by the assumption of crossing over with the additional postulate that secondary linkage groups exist within the polygenic complex that produces the B type. These secondary linkage groups would have the approximate value of 4, as compared with the units of the A complex.

The secondary linkage, which I have postulated, could also provide an explanation of the physiological differences between B and A individuals. With this assumption, the $B^1$ animals may be con-

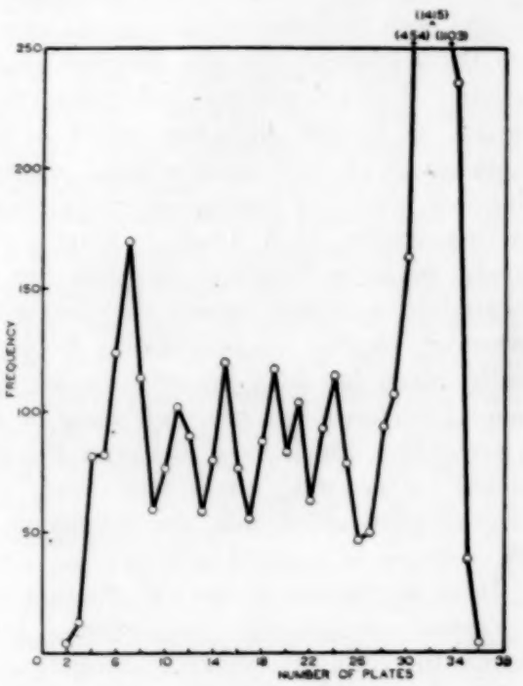


FIG. 11. The frequency distribution of plate numbers in a compound sample of all known B-populations (from Heuts, 1947).

sidered to possess one secondary linkage group; the A animals have no secondary groups. If this is true, it would be expected that in the cross $B^1 \times B^1$ or the cross $B^1 \times A^m$ a recombination leading to the appearance of some animals carrying intermediate plate numbers by crossing-over would be much more probable than recombination toward higher plate numbers in an $A^m \times A^m$ cross. Only one cross, between low-plated fishes, wherein a presumed secondary linkage group is involved, has thus far been made, and yet the expected recombinations toward the intermediate plate numbers took place (figure 10, g.). The number of representatives of the intermediate types (caudal crest) was 5 out of 50 fishes. A mechanism of this sort would explain the physiological differences between $A^1$ and $B^1$ fishes, if one assumes that the threshold value of one secondary linkage group produces a sharp shift in physiological properties. It is probable that this is responsible for the divergence and maintenance of the two types.

## Discussion

Although the observational and experimental data reported in the present article are based entirely on the Belgian populations of *Gasterosteus aculeatus,* they permit an insight into the origin of the morphological and geographical discontinuities observed among the populations of this species as a whole. This is not solely because Belgium happens to be located in a region where the biological meaning of the discontinuities is especially clear, but also because the experimental evidence has revealed some of the correlations which exist between the external structural characters and the physiological traits that are important in the process of natural selection.

It is impossible to decide whether the ancestral stickleback populations from which the living ones are descended were adapted for life in fresh water, or in salt water, or in waters of intermediate salinities. Whatever the original materials for evolution in this species may have been, it is reasonable to assume that the ancestral populations carried balanced polygenic complexes that contained plus and minus modifiers of the physiological characters of this fish. These plus and minus modifiers were probably scattered over the whole set of chromosomes. A panmictic population of this kind would have a certain frequency distribution of plate numbers, with a certain mean and with some individuals deviating from this mean in either direction. If one assumes that the physiological characters that adapt the fishes to the entire gamut of salinities at any given temperature are correlated with the plate numbers, then the average condition in the primitive population was adapted to a certain biotope. However the few deviating individuals may have been adapted for life in biotopes slightly different from those in which the average representatives of this primitive population were most nearly at home.

Now, the mineral concentration in natural waters increases not evenly but more or less logarithmically as one proceeds from the source of a stream to its mouth. Not far from the sea the water ceases to be fresh and rapidly becomes brackish and salty. Habitats with any one intermediate salinity between fresh and salt water are very scarce. In other words, natural selection favors particularly two groups of genotypes adapted to the two frequently available biotopes. In Wright's terminology, there exist two important "adaptive peaks" available for occupation by organisms like *Gasterosteus aculeatus* (figure 12). Two such groups of genotypes could arise as a result of a process similar to that postulated by Mather (1941) for the establishment of balanced polygenic systems of the sternital hairs in *Drosophila melanogaster.* We may assume that in the case of the stickleback two systems of this kind have arisen, linking low and high plate number genes closely together, and adapting their carriers to low and to high salinities re-

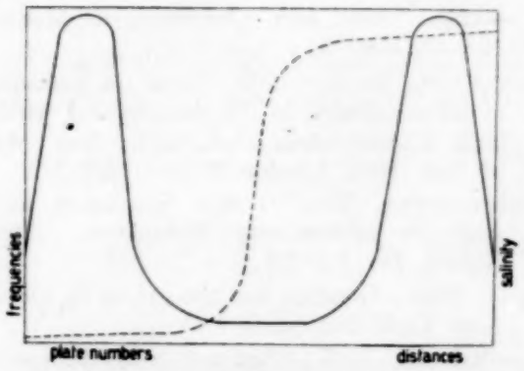


FIG. 12. The bimodal frequency distribution of plate numbers and its adaptation to the salinity gradient in nature. Ordinates—frequencies and salinities; abscissae—plate numbers and distances from the sea.

spectively. However close this linkage may be, crossing-over will produce recombinations adapted for survival at intermediate salinities. If such intermediate habitats are available, the recombination genotypes might serve as gene bridges between the two adaptive peaks. Another mechanism however, presumably a secondary grouping of the genes in the high plate number complex, made these recombination genotypes physiologically more or less alike to one of the extreme types. As a consequence, the intermediate types also became adapted to either one or the other of the two large biotopes. In this way the loss of intermediates through unfitness to the existing conditions is avoided, and at the same time the eventual establishment of gene bridges between the two population groups is prevented.

To be sure, the gap need not to be absolute. Occasional hybridization takes place between the populations of the A and the B types. In fact, it may be advantageous to the populations to preserve some plasticity and adaptability to altered conditions. Some hybridization may permit new mutations or gene combinations possessing high selective values to spread throughout the entire population complex. It is possible that at the present stage of divergence of the stickleback populations this plasticity may have a selective value sufficient to counterbalance the loss of about 50 per cent of eggs that probably occurs when populations of A and B types hybridize. The stickleback populations are split in two types adjusted to a sharp break in the available habitats. This break may, however, become less pronounced following topographic changes and multiplication of the brackish water habitats. Both A and B types would then be at a disadvantage. A channel through which genes may flow between the populations might then facilitate the formation of new gene combinations adapted to the new habitats. Such channels of gene exchange seem to exist between many plant species (Epling, 1947).

With the natural conditions as they are at present, the differentiation of the stickleback populations is distinctly advantageous. This differentiation is maintained despite the fact that migration brings representatives of both types of fish close together at the breeding grounds. The same physiological agents that brought about the differentiation in the past serve to maintain it at present.

## SUMMARY

Populations of the stickleback, *Gasterosteus aculeatus* L., vary greatly in morphological and physiological characters. The populations of Belgium and neighboring countries are differentiated into two sharply defined adaptive types. These types differ in morphological characters, especially in the mean plate numbers and in mean body size, and also in physiological traits, the most important of which concern the chlorine regulation of the blood. In Belgium one type occupies fresh water habitats and the other type salt water habitats. The gene exchange between these types is very limited because of the physiological properties of the hybrid eggs.

Representatives of the same type coming from different countries are often distinct in the mean plate number. These geographical differences form north-south gradients. Indirect evidence is given, based on the study of the type, which in Belgium lives in fresh water, that the

geographical distinctions are adaptive. The establishment of the geographical gradients was in all probability an adaptive process.

An hypothesis is advanced, according to which the two types have arisen through a series of primary and secondary linkage rearrangements starting from an originally single polygenic complex. In this way, two physiologically distinct groups of populations might have been produced. Although these types intergrade morphologically, they are adapted to different combinations of temperature and salinity. The morphological intergrades are not physiologically intermediate between the two types; they belong physiologically, and therefore ecologically, to one or the other type.

The physiological differentiation between and within the two types is large enough to explain the geographical and morphological divergence as well as the maintainance of the two types by natural selection. Natural selection is shown to act not only on the adult stage but even more sharply on the egg stage.

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# GENETIC DRIFT IN A POPULATION OF *DROSOPHILA IMMIGRANS*

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## INTRODUCTION

Mutations, both chromosomal and genic, which first occur in individuals, are recognized by the neo-Darwinists as the "building stones of evolution." However, before such stones can be of value in building the superstructure of progressive or adaptive evolution they must be replicated many times over within aggregates of individuals or populations. The theoretical factors involved in such replication have been presented in a comprehensive series of papers by Wright (1931; 1932; 1942; 1943; 1946). With the development of techniques for the capture and rearing of various species of the fruit-fly genus, Drosophila, and for the analysis of genetic variability within collected samples, in recent years many studies have been made on the genetic structure of Drosophila populations. For a review of this work see Spencer (1947).

In some cases an attempt has been made to correlate the genetic findings with the ecological factors involved in establishing the structure of the populations concerned. As examples of such studies in the U. S. A. we may mention the work of Dobzhansky and his colleagues (Dobzhansky, 1939 and 1943; Dobzhansky, Holz and Spassky, 1942; Dobzhansky and Queal, 1938; Dobzhansky and Wright, 1941), and of Ives (1945), and in Russia of Dubinin and co-workers (1937), and of Berg (1942a; 1942b). These studies carried out on a large scale may be considered preliminary in that they have opened up new possibilities for productive work, and have led to a better understanding of the mechanics of evolution in this genus.

## PREVIOUS WORK ON DROSOPHILA IMMIGRANS

The present report deals with the genetic structure of a "village" population of *Drosophila immigrans,* and is a supplement to earlier studies made by the author on populations of this species. Spencer (1940) presented a résumé of the biology, cytology and genetic information then available on the species and reported on the analysis of the genetic variability of samples of five populations, two from California, and one each from Ohio, Tennessee, and Massachusetts.

As pertinent to our present study it may be stated that each population sample showed its own peculiar constellation of mutant genes, and that as far as could be determined from the admittedly small samples no one mutant gene had a high frequency in any population. This species is one of those introduced into the United States, and forms "island" populations in villages, towns, or even around isolated human habitations. Like *Drosophila melanogaster, simulans, hydei* and several other species it has a tremendous seasonal fluctuation in population size. In the northern U. S., particularly where the summer breeding focus is of small area as in a village or on a fruit farm, such populations may be reduced to a few individuals over-wintering in-doors. From the spotty nature of summer collection records presumably many small island populations may become extinct in severe winters, while others approach this condition. But, given a summer with adequate rainfall, and particularly an autumn with abundant food supplies in tomato patches and orchards, the local

population may reach a number of many thousands of individuals.

## The 1944 Population Sample

On September 11th, 1944, a collection of *Drosophila immigrans* was made from three open traps, baited with ripe tomatoes, and set a few yards apart in a woodland plot near a stream in the village of New Wilmington, Lawrence County, Pennsylvania, a few miles from the Ohio-Pennsylvania border.

In the laboratory 55 pairs of these flies, each pair in a separate culture vial, produced offspring. From each of these broods either 7 or 8 pair-matings were made up. The $F_1$ and $F_2$ flies were all carefully examined under the binocular microscope for mutant types. The results of this analysis have been reported by the author (Spencer, 1946). By the breeding procedure outlined above most

Table 1. *Chance of recovering an autosomal recessive visible present in heterozygous form in one fly of a $P_1$ pair by rearing one to seven $F_1$ pair matings.* Chance expressed in per cent.

| No. $F_1$ cultures | Chance of recovery in $F_2$ expressed in per cent |
|---|---|
| 1 | 25 |
| 2 | 44 |
| 3 | 58 |
| 4 | 68 |
| 5 | 76 |
| 6 | 82 |
| 7 | 87 |

Table 2. *Analysis of 55 $P_1$ pairs of* D. immigrans *from New Wilmington, Pa., by 7 $F_1$ pair matings each. 1944 sample*

Mutants appearing in $F_2$ cultures shown.
Only those numbered $P_1$ pairs tabulated from which mutants were recovered.

| $P_1$ | $F_1$-1 | $F_1$-2 | $F_1$-3 | $F_1$-4 | $F_1$-5 | $F_1$-6 | $F_1$-7 |
|---|---|---|---|---|---|---|---|
| 2 | — | — | — | brick | — | — | — |
| 4 | — | — | eveinless | -- | — | brick | — |
| 5 | minute | — | stubble | — | minute | — | — |
| 6 | — | — | — | stubble | — | — | stubble |
| 9 | — | brick | — | — | — | — | — |
| 15 | — | dubonnet | — | — | stubble | — | — |
| 17 | — | — | brick | — | brick | — | — |
| 18 | — | — | stubble | stubble | — | — | stubble |
| 19 | brick | brick | — | — | — | — | — |
| 21 | — | — | — | — | — | stubble | — |
| 22 | — | stubble | brick | stubble | — | — | — |
| 24 | — | — | — | stubble | stubble | — | — |
| (26) | stubble | — | stubble | (stubble) | stubble | — | — |
| 28 | — | — | — | — | sepia-spineless | — | — |
| 29 | — | brick | — | — | — | — | — |
| 30 | stubble | stubble | — | — | — | — | — |

TABLE 2.—*Continued*

| $P_1$ | $F_1$-1 | $F_1$-2 | $F_1$-3 | $F_1$-4 | $F_1$-5 | $F_1$-6 | $F_1$-7 |
|---|---|---|---|---|---|---|---|
| 32 | broken | stubble | broken stubble | broken | — | broken | purple-net-short |
| 33 | — | — | — | — | stubble | stubble | — |
| 34 | — | — | tiny | — | — | tiny | tiny |
| 35 | — | — | — | — | — | — | stubble |
| 36 | — | dubonnet | — | — | — | dubonnet | — |
| 38 | — | — | — | — | brick | — | — |
| 40 | stubble | — | — | — | stubble | — | stubble |
| 42 | double | — | — | double | — | double | — |
| 43 | — | — | stubble | — | stubble | — | stubble |
| 46 | 2-brist. | 2-brist. stubble short-5 | — | stubble | 2-brist. short-5 | short-5 | short-5 |
| 47 | — | stubble | — | — | — | stubble | — |
| 48 | — | — | small | — | — | — | — |
| 51 | stubble | — | — | purplish-thin-sing. | — | stubble | stubble |
| 52 | — | stubble | stubble | — | — | — | — |
| Total | 9 | 13 | 12 | 10 | 12 | 10 | 9 |

Note: Both parents of $P_1$ (26) were heterozygous for stubble. Both parents of $F_1$26–4 were homozygous stubble.

of the recessive visible mutations carried in heterozygous form in the wild flies could be recovered in the $F_1$ generation if both wild parents were heterozygous for the same mutant factor, and in the $F_2$ if only one of them were heterozygous for the factor. If one of the wild parents were heterozygous for a recessive autosomal mutant gene (m) and the other did not carry this gene, then the $P_1$ mating would be Mm × MM and approximately half the offspring of the mating would be Mm. However, with m recessive all the $F_1$ flies would be wild-type in appearance. In making up an $F_1$ pair-mating the chance of it being of the favorable type, Mm × Mm, to give some homozygous mm offspring among the $F_2$ progeny would obviously be $\frac{1}{4}$. The chance that the mating be of the unfavorable type would therefore be $\frac{3}{4}$. For two pair matings the chance that both be unfavorable would be $(\frac{3}{4})^2$ and for n matings $(\frac{3}{4})^n$. For n matings the chance that at least one be favorable would then be $1 - (\frac{3}{4})^n$. The expectation of recovering the mutant in the $F_2$ if present in one of the wild grand-parents, when 1 to 7 pair-matings of the $F_1$ were made up, is shown in Table 1.

The visible mutations recovered in the $F_2$ cultures are shown in Table 2. It will be seen that a gene for "stubble" bristles was recovered many times, was therefore

present in many of the wild flies in the sample. On the basis of the data in tables 1 and 2 it can be calculated that the gene, "stubble," recovered in the first 5 pair matings from 17 of the 110 wild flies tested, was probably present in about 22 of them. This means that one fly in five carried stubble and as each fly carries two genes at the stubble locus that about 10 per cent of the genes at this locus were stubble and 90 per cent non-stubble or wild-type. The eye color mutant, "brick," was recovered from 7 of the 110 flies in the first 5 pair matings, was therefore presumably present in about 9 flies or with a gene frequency of 4 per cent. Another eye color, "dubonnet," was found twice. Eleven other mutants, of which 3 affected wing veins, 5 were bristle changes, and 3 phenotypic complexes having polymorphic expression were each found once. Cross-tests of the stubble mutants recovered indicated that the several stubble genes recovered were actually identical or indistinguishable alleles. Similar tests on brick established the same fact for this locus.

The high frequency of the stubble gene in the New Wilmington population of 1944 was interpreted by the author (Spencer, 1946) as probably due to genetic drift, the accumulation of genes for stubble through sharp reductions in the winter populations in previous years, with the chance survival and subsequent increase in frequency of a disproportionately large number of stubble genes. As a less likely alternative it was sug-

TABLE 3. *Analysis of 55 $P_1$ pairs of* D. immigrans *from New Wilmington, Pa., by 5 $F_1$ pair matings each. 1946 sample*

Mutants appearing in $F_2$ cultures shown.
Only those numbered $P_1$ pairs tabulated from which mutants were recovered.

| $P_1$ | $F_1$-1 | $F_1$-2 | $F_1$-3 | $F_1$-4 | $F_1$-5 |
|---|---|---|---|---|---|
| 1 | dubonnet | spineless | dubonnet | spineless | |
| 4 | | stubble | | | |
| 6 | | | | stubble | |
| 8 | curled | | stubble | | |
| 10 | | two-bristle | two-bristle stubble | | |
| 11 | | | | net | |
| 12 | spread-wing | | | spread-wing | |
| 13 | dubonnet | dubonnet | | dubonnet | |
| 16 | short-5 | short-5 ragged-vein | short-5 | curled | |
| 17 | pin | | | | |
| 18 | curled | curled | curled | | |
| 21 | | | | stubble | |
| 22 | | | | stubble | stubble |
| 23 | stubble | | | stubble | cut-echinus |

TABLE 3.—*Continued*

| $P_1$ | $F_1$–1 | $F_1$–2 | $F_1$–3 | $F_1$–4 | $F_1$–5 |
|---|---|---|---|---|---|
| 26 | | | | | small-brist |
| 29 | broken x-vein | brick | | broken-x-vein | brick |
| 32 | (miniature) | curled | | | |
| 34 | | brick | brick<br>plexus | plexus | |
| 35 | stubble | | | stubble | |
| 37 | | | stubble<br>fat | stubble | dubonnet |
| 40 | | | | stubble | |
| 47 | curled | curled | | | |
| 49 | ragged-tiny | | | | ragged-tiny |
| 51 | brick | | | | |
| 52 | curled<br>slant | curled | curled | curled | |
| 53 | dubonnet | | stubble | stubble | |
| Total | 16 | 12 | 12 | 16 | 6 (8) |

Note: 13 of the 55 $F_1$ pairs for column 5 failed to give offspring. $F_1$–1 from $P_1$–32 gave some miniature males, a new mutation not present in either $P_1$ parent.

gested that heterozygous stubble might have had a selective advantage over wild-type. A third alternative was suggested, namely that the sample of flies, taken at the same time and in one place in the village, might have contained a large number of offspring from one or a few flies which had carried stubble. On this view the collection would be considered, not as a random sample of the *Drosophila immigrans* population of the village, but rather as a sample of a small local and highly inbred segment of the population. If this were the explanation it would be quite probable that stubble would not survive the following winter and would be lost from the village population, or at least would not occur in high frequency in subsequent years. A more detailed analysis of the 1944 population will be found in the publication referred to above (Spencer, 1946).

## THE 1946 POPULATION SAMPLE

To gain further information on the genetic structure of the New Wilmington population and to test the alternative explanations of stubble gene frequency, a sample of *Drosophila immigrans* was collected from the New Wilmington population on August 31–September 2, 1946. This time the traps were similarly placed within a few yards of one another and baited with tomato, but the collection was made in a garden at a point a little less than ¼ mile distant from the place where the 1944 sample was taken. Again 55 pairs of wild flies were tested and the identical inbreeding technique used, except for the fact that 5 instead of 7–8

TABLE 4. *Comparison of mutations recovered from the 1944 and 1946 samples.* Number after each mutation denotes actual number of recoveries and not the calculated number of genes present in the sample. Larger total number of mutations recovered from 1944 sample due to larger number of $F_2$ cultures reared.

| 1944 | 1946 |
|---|---|
| Eye Colors | Eye Colors |
| brick 8 | brick 3 |
| dubonnet 2 | dubonnet 4 |
| Wing Veins | Wing Veins |
| broken 1 | broken x-vein 1 |
| cross-veinless 1 | plexus 1 |
| short-5 1 | short-5 1 |
| | slant 1 |
| | net 1 |
| | ragged 1 |
| Bristles | Bristles |
| double 1 | pin 1 |
| minute 1 | ragged-tiny 1 |
| small 1 | small bristle 1 |
| stubble 19 | spineless 1 |
| tiny 1 | stubble 11 |
| two-bristle 1 | two-bristle 1 |
| Wing Shape | Wing Shape |
| | curled 6 |
| | cut-echinus 1 |
| | spread wing 1 |
| Body Shape | Body Shape |
| | fat 1 |
| Phenotypic Complexes | Phenotypic Complexes |
| purple-net-short 1 | |
| purplish-thin-singed 1 | |
| sepia-spineless 1 | |
| Total 40 | 38 |

pair-matings of the $F_1$ were made. The results of this test are shown in table 3. The data indicate that again stubble showed a high gene frequency in the population sample. It was recovered from 11 flies and by calculation was probably present in about 15 of the 110 flies tested. The gene frequency of stubble in the 1946 population was therefore about 7 per cent. Cross-tests of the 1946 stubble to a stock of the 1944 stubble showed that the gene was the same and not a mimic. Brick and dubonnet, the two other genes found more than once in the 1944 collection, were again present in the 1946 sample. Their peculiar phenotypes, particularly in the case of dubonnet, which was semi-sterile, left little doubt that the corresponding genes in the two collections were identical. However, cross-tests could not be made as stock of the 1944 mutants had not been saved. The data from the samples analyzed indicated a fall in the gene frequency of brick from about 4 per cent to 1.8 per cent and a rise in the gene frequency of dubonnet from about 1.2 per cent to 2.4 per cent in the two year period. However, the samples are too small to give accurate estimates on gene frequencies of this order of magnitude. Curled, a mutant not found in the 1944 sample, was recovered 6 times in the 1946 sample, giving it a gene frequency of about 3.5 per cent.

Table 4 presents a comparison of the mutations recovered in the 1944 and 1946 samples. These are listed according to their phenotypic effects. Calculations from the analysis of the two samples would indicate that the 110 flies of the 1944 collection carried about 47 visible mutant genes, of which about 33 were at the brick, dubonnet, and stubble loci, and that the 110 flies of the 1946 collection carried about 51 visible mutant genes, of which about 24 were at these same loci.

## DISCUSSION

It seems clear from the analysis of these two samples of equal size taken at the same season of the year and in the same way, but separated in space by almost $\frac{1}{4}$ mile and in time by two years, that the genes stubble, brick, and dubonnet had gained a relatively high frequency in the *Drosophila immigrans* population of this Western Pennsylvania village.

When we consider the breeding structure of *Drosophila immigrans* populations in this latitude, the most reasonable explanation of the high frequency of these genes is that of genetic drift, fluctuations due to chance rather than any concentration of genes due to high mutation frequency or selective advantage. The overwintering population is cut down to a

small number of fortunate individuals, living in fruit-cellars or other favorable refuges, while their thousands of less fortunate neighbors have perished with the onset of cold weather. If, in the spring, an impregnated female reaches a favorable store of food, she may lay 100 or more eggs here. Even if other females reach this store of food within a few days, the brood from the first female will emerge earlier and have a tremendous advantage in conditioning the genetic structure of the population rapidly building up toward a maximum under conditions when food is abundant and selective competition low. If the first female referred to above happened to carry a gene such as stubble, this gene would thus be spread in the population even though it had no selective advantage. In a series of such winter bottle-necks the same gene might by chance be favored more than once and its spread through the population assured. It must be remembered that many other mutant genes are present in the population; while the chance of any one of them, chosen at random, being so favored is small, some gene such as stubble might well be the fortunate one, while many others are being eliminated or reduced in frequency. Once a gene reaches a high frequency it may then persist for many years in appreciable numbers in the population. Sooner or later the same mechanism which brought about its rise in frequency will probably result in its reduction in numbers or even its eventual elimination.

We may now enquire what bearing the rise in frequency of a gene such as stubble has on the evolution of the local population and on the species. The answer in this particular case, as we have no indication that stubble itself has any selective advantage, is that it may have no direct bearing on evolution. However, the demonstration that mutant genes may gain high frequencies in a population by the mechanism of genetic drift does indicate the possible role which this mechanism may play in progressive evolution. If, as Wright has postulated, favorable gene combinations form adaptive peaks leading to a better adjustment of the organism to its environment and thus to progressive evolution, genetic drift may play a considerable role in concentrating the gene constituents which enter into the formation of such adaptive peaks, and in increasing the chance of such peaks being formed on a scale large enough that they will not immediately be worn away by the weathering effects of accident, the unfortunate elimination of favorable adaptive combinations when in too low concentrations. It seems patent that there is a constant interplay between genetic drift and selection of favorable combinations. Neither of the two factors is likely to operate alone. The relative roles may differ in individual cases, but both factors contribute to the primary steps leading to the origin of new sub-species and species.

Along with adaptive characters there must be evolved incipient isolating mechanisms; it would seem that genetic drift might well concentrate the constituent genes of such isolating mechanisms in sufficient numbers so that they could gain a foothold in some spatially isolated section of the species, an island population. It should be emphasized that the concentration of specific genes by genetic drift may be greatly facilitated by the reproductive advantage of one or a few favored individuals, who happen to reach an untapped food store in the short-time fluctuating seasonal cycles. The magnitude of this factor will, of course, differ from population to population and from season to season. It seems probable that such short time oscillations play a more significant role in the evolution of some organisms than has generally been supposed by those workers who have concentrated attention on the role of selection as a principal factor in evolution at the level of incipient speciation.

## Summary

The analysis of a sample of 110 wild flies from the September, 1944, population of *Drosophila immigrans* in the village of New Wilmington, Western Pennsylvania, showed a frequency of 10 per cent for the gene, "stubble" bristles. In a sample of identical size, collected at a point almost ¼ mile distant from the first collection area and in September, 1946, the gene frequency for stubble was 7 per cent. The genes, "brick" eye color and "dubonnet" eye color, were also recovered more than once in both samples. The concentration of these genes in this "island" population is best explained by genetic drift brought about by seasonal fluctuations in population size. The role of the interaction of this factor with selection in establishing favorable adaptive combinations is discussed.


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# NOTES AND COMMENT

## A NEW METHOD OF TREATING DROSOPHILA GAMETES WITH CHEMICALS

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Studies on induction of mutations by chemical substances have two aspects. First, the establishment that a given chemical is mutagenic; second, the analysis of its mode of action. The success at either stage depends on the sensitivity of the techniques used in the production and detection of inheritable changes. If the mutagenic chemical substance employed is highly efficient in affecting genes, it may matter little what kind of organism or cell is chosen for treatment, so long as there is facility in handling large numbers of individuals and in detecting mutants that may be induced. On the other hand, if the chemical is not very efficient and is intrinsically weakly mutagenic, it may well matter just what organism or cell is treated; an improper choice may yield deceptive negative results. Caution is necessary, for chemicals that are weakly mutagenic may be as important as, and conceivably more important, than highly mutagenic compounds in the elucidation of the origin and the mechanisms of spontaneous mutation, as well as the modes of gene action and reduplication.

It would appear that sperm cells offer one of the most sensitive materials for testing chemicals for mutagenic action. If a substance is at all mutagenic then it is most likely to produce an effect in a sperm cell. This is because sperm cells possess a minimum of cytoplasmic or metabolic materials that may serve as insulation against changes in the hereditary material. Specifically, the spermatozoa of Drosophila are very suitable for detection of mutations; they are especially favorable because the inherited changes may be subsequently accurately localized in the chromosomes and compared with those produced by other mutagenic agents.

Treatment of sperm suspensions with chemicals followed by artificial insemination would seem to be the most direct technique of testing for mutagenic action. Unfortunately, artificial insemination has not yet become feasible in Drosophila. Several methods are already known that indirectly circumvent this difficulty by exposure of adult flies to gases or areosols (3) (4). A new technique, which permits direct treatment of the spermatozoa, seemed nevertheless desirable. Accordingly, an investigation was initiated to discover if chemicals could be injected into the vagina of adult females, which would then copulate, and thereby expose the sperm to the respective chemicals.

Virgin females of the Muller-5 * stock of *Drosophila melanogaster* were aged for 2 to 5 days, lightly etherized and placed under a binocular microscope (mag. 23 ×) for injection. A female was forced to expose her vaginal opening by means of compressing her abdomen laterally with an ordinary coarse forceps. A micropipette, attached to a ½-cc. syringe, mounted on a Taylor type micromanipulator, was inserted into the vaginal opening and the vagina flooded with the liquid. For copulation the freshly injected females were placed individually in vials with several males of the Ore-R wild type stock, previously aged for 4 to 9 days. A single copulation was permitted, after which the males were killed and the females placed individually in small containers ("creamers") with culture medium. Only females which mated within two hours after injection were investigated. Transfers to fresh creamers were made every three days until no further offspring were produced. Saline, prepared according to Buck and Melland (2), was employed as a solvent or suspension medium for the chemical substances used in all injections.

Preliminary experiments with India ink indicated that particles of the ink remained in the vagina of virgin females for some hours after injection. It was also observed that after copulation the sperm in the vagina was in contact with India ink particles. Thus, the actual contact of sperm with the chemical was demonstrated. It is interesting to note that no India ink particles were ever observed in the ventral receptacle, even after the sperm had entered there, having first mixed with the ink in the vagina. This strongly suggests that spermatozoa normally enter the receptaculum by means of their own motions.

To test the efficiency of the technique for detection of mutagenic action, injections were made of methyl bis (b-chlorethyl) amine hydrochloride at concentrations of 0.2% and 10.0%. Lethals produced in the X chromosomes of the

* Stocks used were kindly supplied by Dr. M. Demerec.

spermatozoa were detected in the $F_2$ and retested in a subsequent generation by usual methods. Untreated controls were not considered necessary in view of the low rate of spontaneous mutability known for the Ore-R stock (4). The results observed are summarized below:

| Concentration | No. females injected | No. females fecund | No. sperm X chromosomes tested | No. lethals |
|---|---|---|---|---|
| 0.2% | 62 | 24 | 867 | 4 |
| 10.0% | 25 | 9 | 127 | 7 |

Two gynandromorphs were observed in the 0.2% and one in the 10.0% treatments. All three were largely female, which supports the hypothesis of delayed effects of mustard compounds (1). In addition, 113 X chromosomes coming from the eggs of the treated females were tested in the 10.0 per cent treatment, and 4 lethals were detected. How this highly interesting effect on the chromosomes of the eggs is produced by the chemical injected into the vagina has not yet been determined.

The data presented above establish the practicality of the vaginal douche technique for the detection of chemical mutagens. There are two advantages of this method. Low concentrations of mutagens may be effective because of the direct contact of the chemical with the sperm; high concentrations of chemicals can be used without killing the organism, since a localized part of the body rather than the whole individual is treated. These advantages point to the possibility of a chemical analysis of the processes leading to gene changes.

I wish to express my appreciation and gratitude to Dr. Th. Dobzhansky for his many helpful suggestions and to Dr. T. Hayashi for his encouragement and invaluable cooperation throughout the course of this investigation.

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